ANNUAL RESEARCH PROGRESS REPORT

(FY 2005)

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHERN PLAINS AREA

GRAND FORKS, NORTH DAKOTA 58202

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GRAND FORKS, NORTH DAKOTA 58202

NUTRITIONAL DETERMINANTS OF HEALTH

MANAGEMENT UNIT

5450-010-00

Project Number: 5450-51000-038-00D **Accession:** 0408766 **FY:** 2005
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: DAVID M KLURFELD
Start Date: 07/21/2004 **Term Date:** 04/30/2009
National Programs: 107 N Human Nutrition
Title: DIETARY COPPER REQUIREMENTS FOR OPTIMAL CARDIOVASCULAR FUNCTION AND HEALTH
Period Covered From: 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?**

Cardiovascular disease is the leading cause of death in this country, with the direct annual cost projected to approach $250 billion in 2005. Basic research and epidemiological studies have indicated that inadequate dietary intakes of mineral elements such as calcium, copper, magnesium, zinc, as well as overload of iron are associated with altered functions of the heart and circulation. Furthermore, dietary surveys indicate that appreciable numbers of people have sub-optimal intakes of at least some of these minerals. However, we presently have little definitive proof that changing dietary practices with regard to these minerals will benefit cardiovascular health. A clear understanding of how these minerals, particularly at marginal intakes, contribute to cardiovascular function will provide the basis for dietary recommendations that improve the health of the general public. The current project will focus on the contribution of dietary copper to cardiovascular health.

The overall objective is to determine, using animal models, whether copper (Cu) intakes consistent with those observed in humans can adequately support cardiovascular functions. This objective will be pursued through the mechanistic tether of oxidative stress/altered nitric oxide metabolism by which Cu functions, and includes the following specific objectives 1) to develop a strategy for assessment of marginal copper deficiency in animals; to use this strategy to determine biomarkers of copper status that are suitable for assessment of marginal status in humans, 2) to determine the contribution of oxygen- and nitrogen-derived reactive species to the cardiomyopathy (metabolic, contractile) induced by Cu deficiency, and the dietary intakes at which this pathology occurs, 3) to determine whether low Cu intakes consistent with those observed in humans can impair nitric oxide-dependent control of blood vessels and blood pressure regulation, 4) to determine whether the oxidative stress induced by Cu deficiency affects homocysteine metabolism and, thereby, cardiovascular function, and whether such effects influence nitric oxide-dependent signal transduction and/or other mechanisms that affect atherosclerosis, 5) to determine whether marginal Zn deficiency can exacerbate or unmask cardiovascular effects of sub-optimal Cu status by virtue of its role in oxidative/nitrosative metabolism.

Research will address components of National Program 107, Human Nutrition (100%). From Component 1, Nutrition Requirements, Objectives A (Biomarkers), B (Mechanism of Action), C (Nutrient Interactions), E (Genetic Variability) and G (Function and Performance) will be addressed. From Component 2, Diet, Genetics, Lifestyle, and the Prevention of Obesity and Disease, Objective A (Identify nutritional, environmental and genetic factors that modify the effects of nutrient

intake and metabolism on health outcomes) will be addressed.

2. **List the milestones (indicators of progress) from your Project Plan.**

Year 1 (FY 2005)

Assess relationships between organ copper and marginal intakes of dietary copper; select organ with best discrimination of copper intake.

Determine if copper deficiency causes reduced mitochondrial respiratory complex activity.

Identify mitochondrial respiratory complexes causing increased hydrogen peroxide production during copper deficiency.

Determine nitric oxide effect on mitochondrial respiration in copper deficiency.

Determine effect of copper deficiency on homocysteine metabolism.

Year 2 (FY 2006)

Determine signaling pathway for induction of inducible nitric oxide synthase during copper deficiency.

Determine nature of effect of altered nitric oxide on blood pressure during copper deficiency.

Determine effect of copper deficiency on bilirubin and biliverdin reductase.

Determine if low zinc acts to exaggerate cardiovascular effects of marginal copper.

Year 3 (FY 2007)

Correlate organ copper content with semi-direct indicators of copper status; select best potential single biomarker(s).

Identify respiratory complexes affected by developmental copper deficiency.

Determine extent of oxidative modification of mitochondrial DNA by copper deficiency.

Determine role of nitric oxide in impaired contractile function of copper deficiency.

Determine relationship between nitric oxide, oxidative stress, homocysteine in atherosclerotic symptoms of copper deficiency.

Clarify role of oxidative/nitrosative stress in zinc/copper interaction.

Year 4 (FY 2008)

Determine nature of effect of altered nitric oxide on coronary vessels during copper

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deficiency.

Determine role of hemoxygenase in atherosclerotic effects of copper deficiency.

Year 5 (FY 2009)

Correlate organ copper content with combinations of indicators of copper status; select best combination of indicators as biomarker.

Identify oxidized, nitrated mitochondrial proteins in copper deficiency.

Identify mitochondrial DNA mutations of copper deficiency.

Relate mitochondrial DNA mutations to expression of respiratory complexes caused by copper deficiency.

[Determine whether elevation of inducible nitric oxide synthase preconditions copper-deficient hearts (contingency).]

3a. List the milestones that were scheduled to be addressed in FY 2005. For each milestone, indicate the status: fully met, substantially met, or not met. If not met, why.

1. Assess relationships between organ copper and marginal intakes of dietary copper; select organ with best discrimination of copper intake.

   Milestone Substantially Met

2. Determine if copper deficiency causes reduced respiratory complex activity, and Identify respiratory complexes causing increased hydrogen peroxide.

   Milestone Substantially Met

3. Determine nitric oxide effect on mitochondrial respiration in copper deficiency.

   Milestone Not Met

   Reason not met: Progress slowed by resource limitations (human, fiscal, equipment, etc.)

4. Determine effect of copper deficiency on homocysteine metabolism.

   Milestone Not Met

   Reason not met: Other

5. Determine whether dry edible beans can supply copper to reverse indices of copper deficiency (pilot to test1).

   Milestone Substantially Met

3b. List the milestones that you expect to address over the next 3 years (FY 2006, 2007, and 2008). What do you expect to accomplish, year by year, over the next 3 years under each milestone?

Year 1 (FY 2006)

Determine signaling pathway for induction of inducible nitric oxide synthase.

Examine effects of copper deficiency on protein levels, mRNA, transcription and activation factors for isoforms of nitric oxide synthase in hearts of laboratory animals. The approach on this milestone will be altered somewhat by preceding the animal studies with cell culture techniques that use an immortalized mouse heart cell line and copper chelation techniques. We anticipate that this approach will

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greatly accelerate progress toward identity of altered signalling pathways in copper-deficient hearts. IMPACT: These studies will aid in defining, on a molecular level, the basis for heart failure in dietary copper deficiency.

Determine nature of effect of altered nitric oxide on blood pressure.

Utilize pharmacological inhibition of nitric oxide synthase with L-arginine analogs, in combination with blockade of hormones responsible for blood pressure regulation, to determine the pathways involved in altered blood pressure regulation caused by dietary copper deficiency. IMPACT: Because dietary copper deficiency is known to impair blood vessel function by altered nitric oxide metabolism, this study will examine whether this impairment carries over to alteration of blood pressure.

Determine effect of copper deficiency on bilirubin and biliverdin reductase.

This milestone will not be pursued because of the retirement of the scientist proposing it.

Determine if low zinc acts to exaggerate cardiovascular effects of marginal copper.

A factorially arranged experiment with rats will be performed to determine whether a moderate zinc deficiency is a stressor of copper metabolism or oxidative metabolism involving copper such that it increases the risk of cardiovascular dysfunction in marginal copper-deficiency. IMPACT: Because zinc and copper are known to affect each other's metabolism and because each is known to influence oxidative stress, this study will determine whether these two essential nutrients act synergistically in influencing cardiovascular function.

Year 2 (FY 2007)

Correlate organ copper content with semi-direct indicators of copper status; select best potential single biomarker(s).

Utilizing long-term marginal copper deficiency studies in laboratory animals, correlate organ copper content with candidate indirect measures of copper status such as cytochrome c oxidase in platelets, extracellular superoxide dismutase in plasma, coagulation factors V and VIII, diamine oxidase, copper chaperone for superoxide dismutase, to determine which ones are most sensitive to marginal copper intakes. IMPACT: Without sensitive, non-invasive indicators of copper status, the role of copper in human cardiovascular function is difficult to assess. These studies are aimed at providing candidate biomarkers of copper status for potential use in human studies.

Identify respiratory complexes affected by developmental copper deficiency.

Respiratory complex activities and protein subunits of complex I and complex IV will be assayed in the hearts of neonates from marginally Cu-deficient dams at various ages up to 1 year. IMPACT: Parallel reductions in complex activities and protein subunit content in cardiac mitochondria indicate that marginal Cu intakes during pregnancy leads to prolonged impairment of mitochondria function in the first generation and increases risk for developing age-related cardiac disease.

Determine extent of oxidative modification of mitochondrial DNA by copper deficiency.

The content of 8-hydroxydeoxyguanosine will be measured in heart mitochondrial DNA from rats fed diets containing deficient, marginal, and adequate levels of copper. IMPACT: Increased 8-hydroxydeoxyguanosine indicates that mitochondrial DNA can be oxidatively modified during Cu deficiency. This can cause mutations in mitochondrial DNA that permanently impair mitochondrial function and lead to cardiac disease.

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Determine role of nitric oxide in heart contractile function.

Determine by acute and chronic inhibition of nitric oxide synthase whether nitric oxide plays a role in altered contractile function of hearts from copper-deficient animals. IMPACT: This study is aimed at determining the mechanism of action of low dietary copper in impairing heart function.

Determine relationship between nitric oxide, oxidative stress, homocysteine in atherosclerotic symptoms of copper deficiency.

A series of experiments will be initiated that will be aimed at determining the relative effects and interactions between nitric oxide, oxidative stress and homocysteine in causing atherosclerotic vessel damage in marginally copper-deficient animals. IMPACT: This study will examine the molecular mechanism of the now well-established impairment of blood vessel function caused by dietary copper deficiency.

Clarify role of oxidative/nitrosative stress in zinc/copper interaction.

If it is determined that a moderate zinc deficiency exacerbates signs of marginal copper deficiency, an experiment with rats will be performed to determine whether cardiovascular effects are modified by changing nitric oxide formation. IMPACT: If, in the year 2006 study, zinc proves to have an effect on copper-dependent alteration of cardiovascular function, a nitric oxide mechanism will be tested to further our knowledge of the molecular events affected by these two nutrients.

Year 3 (FY 2008)

Determine nature of effect of altered nitric oxide on coronary vessels during copper deficiency.

Hearts of animals fed varying levels of dietary copper will be isolated and examined for coronary blood vessel response to nitric oxide donors, selective nitric oxide synthase inhibitors, antioxidants and nitric oxide scavengers to assess whether copper deficiency causes pathologic dysfunction in coronary vessels via reactive oxygen or nitrogen species is it does in systemic blood vessels. IMPACT: The well known alteration of blood vessel function in the peripheral circulation will be examined directly in blood vessels of the heart. This will help to determine whether the known effect of copper deficiency on heart cells is caused by or exacerbated by an effect on the heart's circulation.

Determine role of hemoxygenase in atherosclerotic effects of copper deficiency.

Rats of varying copper status will be subjected to carotid artery damage and then treated with inhibitors of heme oxygenase (metalloprotoporphyrins) to determine the extent to which this enzyme plays a role in the atherosclerotic effects of dietary copper deficiency. IMPACT: This study will further delineate the mechanism of action of low dietary copper in impaired blood vessel function.

4a. What was the single most significant accomplishment this past year?

Long-term marginal copper deficiency in adult animals causes heart and blood vessel pathology.

Dietary surveys indicate that many humans consume less than the recommended amount of copper. Although studies in animals indicate that dietary copper deficiency causes defects in cardiovascular (heart and blood vessel) structure and function, copper intake in these studies is generally too low to be relevant to human consumption. In a collaborative study with the University of Louisville, scientists at the GFHNRC found that if adult rats are fed marginally-deficient levels of copper, but for sufficiently long periods of time, they too exhibit defects in cardiovascular function and structure that are similar to those observed

in severely-deficient young rats. IMPACT: These findings, reported in two papers (Falcone et al, 2005; another in press), illustrate that low copper intakes in animals that are comparable to those observed in humans can impair heart and blood vessel function. This provides a rationale for testing for such a possibility in humans.

4b. List other significant accomplishments, if any.

Pinto beans provide a bio-available source of dietary copper.

Dry edible beans have been shown to be beneficial to cardiovascular health, but the active ingredient(s) for this benefit is(are) unknown. Because copper is known to be essential for cardiovascular health and beans are known to be a good source of copper, we hypothesize that copper from beans could support cardiovascular health. In order to determine this we first tested whether the copper from dry edible beans was bio-available. We found that when copper was fed to copper-deficient rats in the form of pinto beans, it was equally as effective as inorganic copper in restoring copper status indices (e.g., organ copper, activity of copper-dependent enzymes) to those rats. IMPACT: These findings, being prepared for a meeting presentation, indicate that the bean's structure does not impede the delivery of its copper to the animal, which paves the way for testing of effectiveness of bean copper on cardiovascular function in both animals and humans.

Copper deficiency causes elevation of a structural elastic protein in the heart.

We have demonstrated for the first time that there is an 85% increase in fibulin-5 (also known DANCE/EVEC) and a 71% decrease in cytochrome C oxidase (CCO) VIb subunit, but no change in succinate dehydrogenase complex (also known complex II) IP subunit in Cu-deficient rat heart when compared with that of Cu-adequate rats. IMPACT: The elevation of fibulin-5, in particular, is important, because it implies that survival mechanisms have been initiated, which indirectly confirms that dietary copper deficiency leads to heart failure.

4c. List any significant activities that support special target populations.

None.

4d. Progress report.

None.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

[This report is the first for this CRIS project (certified in 2004), thus the accomplishments for the life of the project are incorporated in sections 4a and 4b. The following are related accomplishments that immediately preceded this CRIS project and thus provide background and continuity to the studies of the project.]

Copper deficiency causes signs of heart failure.

Although many contractile, electrical and metabolic abnormalities have been found in hearts of copper-deficient animals, characteristics that unequivocally indicate heart failure have not been demonstrated. Scientists at the Grand Forks Human Nutrition Research Center, in collaboration with scientists at the University of Louisville, measured variables in hearts of copper-deficient mice that are used in humans to demonstrate heart failure. Hearts of copper-deficient mice were found to have reduced maximum contractile pressure, elevated pressure during relaxation, reduced responsiveness to excitation by adrenalin and increased collagen deposits, all of which are signs of heart failure. IMPACT: This suggests that dietary copper deficiency is a risk factor for heart disease and subsequent cardiac failure. NP 107 Action Plan Component 4: Nutrient Requirements; ARS Strategic Plan Performance

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Measures 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients in the food supply and provide that information in databases.

Impaired blood pressure regulation in copper deficiency is related to nitric oxide.

Although prior studies have shown that blood vessels of copper-deficient rats respond poorly to agents that stimulate release of nitric oxide, a potent endogenous dilator, it has not been shown that this causes an alteration of blood pressure. In studies at the Grand Forks Human Nutrition Research Center, rats were injected with a drug (L-NAME) that inhibits formation of nitric oxide and elevates blood pressure. The elevation of blood pressure by L-NAME injection was found to be depressed in rats that were fed inadequate copper. IMPACT: This indicates that dietary copper deficiency causes an impairment of blood pressure regulation and a predisposition to high blood pressure. NP 107 Action Plan Component 4: Nutrient Requirements; ARS Strategic Plan Performance Measures 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients in the food supply and provide that information in databases.

Copper deficiency enhances atherosclerosis-like blood vessel damage.

Although dietary copper deficiency has been shown to enhance inflammation, the relationship between dietary copper and blood vessel injury has not been previously examined. The Grand Forks Human Nutrition research Center, in collaboration with collaborators at the University of Louisville, examined effects of injury induced by balloon inflation within blood vessels of copper-deficient and copper-adequate rats. Blood vessel injury by balloon inflation within the vessel caused thickening of the blood vessel wall (akin to atherosclerosis) that was exaggerated by restriction of dietary copper. IMPACT: This finding provides further support for the view that the inflammatory response to injury is exaggerated by copper deficiency and, further, because balloon inflation of blood vessels (angioplasty) is a common mode of treatment for atherosclerotic blood vessels, the findings suggest that proper copper nutrition may improve the results of such treatment. NP 107 Action Plan Component 4: Nutrient Requirements; ARS Strategic Plan Performance Measures 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients in the food supply and provide that information in databases.

Copper deficiency during pregnancy causes long-term effects in offspring.

Women of child-bearing age consume less than the recommended daily requirement of copper, but it is not known if low dietary copper intake during pregnancy has long-term effects on the cardiovascular system of children. To test this possibility in laboratory animals, scientists at the Grand Forks Human Nutrition Research Center bred rats to bear and nurse offspring during maternal copper deficiency; pups were then fed copper-adequate diets for nine months following weaning and assessed for altered heart mitochondrial function, enzymes and oxidative stress. Activities of respiratory complexes of heart mitochondria in neonates from copper deficient dams was significantly reduced at 21 days following birth and could not be restored to normal activity levels by 6 weeks of copper supplementation. Further, despite nine months of copper repletion, rats of copper-deficient dams exhibited an abnormally low activity of cytochrome c oxidase, a copper-dependent enzyme, in heart mitochondria and an increase in heart mitochondrial hydrogen peroxide production. IMPACT: This finding indicates that copper deficiency during pregnancy has long-term, possibly irreversible, effects on energy metabolism in the hearts of neonates

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that could increase their susceptibility to heart disease as adults. NP 107 Action Plan Component 4: Nutrient Requirements; ARS Strategic Plan Performance Measures 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients in the food supply and provide that information in databases.

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**

The usual routine transfer of nutritional knowledge about the nutritional, beneficial, and non-beneficial effects of trace elements was made through direct contact with industry representatives and the public and with other scientists through presentations at national and international meetings and professional publications.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below).**

J.T. Saari wrote an article which appeared in January 2005 in the nutrition section of the Grand Forks Herald entitled "Being Heart Healthy Doesn't Need to be Hard."

W.T. Johnson wrote an article which appeared in June 2005 in the nutrition section of the Grand Forks Herald entitled "Copper - A Building Block for a Healthy Baby."

Scientific Publications: **Log 115:**

1. Johnson, W.T., Newman, S.M., Jr. 2003. Copper deficiency: A potential model for determining the role of mitochondria in cardiac aging. Journal of the American Aging Association. 26:29-38. **0000113973**

2. Klevay, L.M., Christopherson, D.M., Shuler, T.R. 2004. Hair as a biopsy material: trace element data on one man over two decades. European Journal of Clinical Nutrition. 58:1359-1364. **0000139104**

3. Raymond, L.J., Johnson, W.T. 2004. Supplemental ascorbate or alpha-tocopherol induces cell death in Cu-deficient HL-60 cells. Experimental Biology and Medicine. 229:885-894. **0000153839**

4. Zeng, H., Saari, J.T. 2004. Increased type I collagen content and DNA binding activity of a single-stranded, cytosine-rich sequence in the high-salt buffer protein extract of the copper-deficient rat heart. Journal of Nutritional Biochemistry. 15:694-99. **0000157220**

5. Falcone, J.C., Saari, J.T., Kang, Y.J., Schuschke, D.A. 2005. Vasoreactivity in an adult rat model of marginal copper deficiency. Nutrition Research. 25:;177-186. **0000160799**

6. Dong, F., Esberg, L.B., Roughead, Z.K., Ren, J., Saari, J.T. 2005. Increased contractility of cardiomyocytes from copper-deficient rats is associated with upregulation of cardiac insulin-like growth factor-I receptor. American Journal of Physiology Heart and Circulatory Physiology. 289:78-84. **0000170533**

7. Song, Y., Wang, J., Li, Y., Du, Y., Arteel, G.E., Saari, J.T., Kang, Y.J., Cai, L. 2005. Cardiac metallothionein synthesis in streptozotocin-induced diabetic mice, and its protection against diabetes-induced cardiac injury. American Journal of Pathology. 167(1):17-26. **0000179025**

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8. Wang, L., Zhou, Z., Saari, J.T., Kang, Y.J. 2005. Alcohol-induced myocardial fibrosis in metallothionein-null mice: Prevention by zinc supplementation. American Journal of Pathology. 167(2):337-344. 0000181348

9. Zhou, A., Wang, L., Song, Z., Saari, J.T., McClain, C.J., Kang, Y.J. 2005. Zinc supplementation prevents alcoholic liver injury in mice through attenuation of oxidative stress. American Journal of Pathology. 166(6):1681-1690. 0000163066

10. Johnson, W.T. 2005. Copper and brain function. In: Lieberman, H.R., Kanarek, R.B., Prasad, C., editors. Nutritional Neuroscience. Boca Raton, FL:CRC Press/Taylor & Francis Group. p. 289-305. 0000139159

11. Klevay, L.M. 2005. Copper. In: Coates, P.M., Blackman, M.R., Cragg, G., Levine, M., Moss, J., White, J., editors. Encyclopedia of Dietary Supplements. New York: Marcel Dekker/Taylor and Francis Group. p. 133-141. 0000127097

12. Klevay, L.M., Combs, G.F. 2003. Mineral elements related to cardiovascular health. Proceedings of the World Health Organization Workshop, Rome, Italy, November 11-13, 2003. 0000162465

13. Johnson, W.T., Lukaski, H.C. 2005. Serum superoxide dismutase (SOD) activity is an index of copper status [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1485. 0000171169

14. Saari, J.T., Dong, F., Esberg, L.B., Roughead, Z.K., Ren, J. 2005. Increased contractility is associated with increased cardiac IGF-I receptor protein in copper-deficient cardiomyocytes [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1486. 0000171146

15. Schuschke, D.A., Gordon, S.A., Saari, J.T., Lentsch, A.B. 2005. Impaired deformability of copper-deficient neutrophils [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1485. 0000171179

16. Li, Y., Wang, L., Schuschke, D.A., Zhou, Z., Saari, J.T. 2005. Marginal dietary copper restriction induces cardiomyopathy in rats. Journal of Nutrition. 135:2130-2136. 0000181291

17. Gordon, S.A., Lominadze, D., Saari, J.T., Lentsch, A.B., Schuschke, D.A. 2005. Impaired deformability of copper-deficient neutrophils. Experimental Biology and Medicine. 230:543-548. 0000179029

Approved: CHANDLER LAURENCE D **Date:** 09/20/2005

Project Number: 5450-51000-039-05N **Accession:** 0407993 **FY:** 2005
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH

Start Date: 03/01/2004 **Term Date:** 02/28/2009

National Programs: 107 N Human Nutrition

Title: HISTOMORPHOMETRIC AND BIOCHEMICAL ASSESSMENT OF THE POSSIBLE AUGMENTATION OF BONE HEALING AND REMODELING BY BORON

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-4-0038F

Organization Name: UNIVERSITY OF SALTA

Progress and Outcomes:

4d. Progress report.

This report serves to document research conducted under a non-funded cooperative agreement 58-5450-4-0038F between ARS and the National University of Salta, Argentina. Additional details of the research can be found in the reports for the parent CRIS project 5450-51000-034-00D, Mineral Intakes for Optimal Bone and Joint Development and Health.

The purpose of this research is to determine whether boron is bioactive in bone formation and thus promotes bone growth and remodeling. Findings from this research should be useful in providing dietary guidance for bone repair and health. The animal portion of the experiment to determine the effect of boron deprivation on peri-implant bone healing of the tibia, and mandibular bone remodeling upon tooth extraction in mice has been completed. The histological sections of the mandibles of the mice have been completed. The sections are currently undergoing histomorphometric evaluation.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/23/2005

Project Number: 5450-51000-039-06S **Accession:** 0408592 **FY:** 2005
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH
Start Date: 07/16/2004 **Term Date:** 06/14/2006

National Programs: 107 N Human Nutrition

Title: THE NUTRITIONAL ROLE OF BORON IN THE PREVENTION OF DIABETES

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-4-0366

Organization Name: **NORTH DAKOTA STATE UNIVERSITY**

Progress and Outcomes:

4d. Progress report.

This report serves to document research conducted under specific cooperative agreement #58-5450-4-366 between ARS and North Dakota State University. Details of this project can be found in the report for the parent CRIS 5450-510000-034-00D.

To better define the mechanism by which dietary boron decreased plasma insulin levels and increased insulin sensitivity, we have successfully conducted an animal study. The findings were summarized in the parent CRIS and were reported at the VIIth Conference of the International Society for Trace Element Research in Humans in Bangkok, Thailand in November 2004.

Scientific Publications: **Log 115:**

Approved: CHANDLER LAURENCE D **Date:** 09/23/2005

Project Number: 5450-51000-039-07T **Accession:** 0408848 **FY:** 2005
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH

Start Date: 07/01/2004 **Term Date:** 04/30/2006

National Programs: 107 N Human Nutrition

Title: EFFECT OF ARGININE SILICATE INOSITOL COMPLEX ON BONE AND JOINT HEALTH

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 04-5450-4-0415

Organization Name: NUTRITION 21, INC.

Progress and Outcomes:

4d. **Progress report.**

This report serves to document research conducted under a Trust Fund Cooperative Agreement 58-5450-4-0415 between ARS and Nutrition 21, Inc., Purchase, NY. Additional details of the research can be found in the reports for the parent CRIS project 5450-51000-034-00D, Mineral Intakes for Optimal Bone and Joint Development and Health.

The purpose of this research is to determine whether silicon as an arginine silicate inositol complex is useful in overcoming the lack of dietary silicon that may lead to pathological changes in bone or connective tissue including bone cartilage, and thus result in bone erosion or loss. An experiment was performed to determine whether deprivation exacerbates collagen-induced inflammatory arthritis, and any associated bone loss in rats, and this exacerbation is alleviated by silicon supplementation as arginine silicate inositol complex or as sodium metasilicate. Tests in the experiment were oriented towards assessing whether changes in collagen metabolism related to bone and joint status occurred. The silicon supplementation did not alleviate the induced arthritis, nor did it markedly affect collagen-related indicators. However, findings were obtained that indicated bone structure may be affected by silicon through altering glcosaminoglycan metabolism. Another experiment is currently underway to determine whether dietary silicon changes glycosaminoglycan, particularly hyaluronan, formation, structure, or ability such that it affects bone shape and strength.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/23/2005

Project Number: 5450-51530-009-00D **Accession:** 0408299 **FY:** 2005
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH

Start Date: 04/03/2004 **Term Date:** 04/02/2009

National Programs: 107 N Human Nutrition

Title: MICRONUTRIENT ROLES IN PHYSIOLOGY AND HEALTH

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?**

Suboptimal dietary intakes of essential micronutrients have been statistically associated with chronic disorders such as obesity, diabetes, cardiovascular disease, depression and dementia. Further, national nutrition surveys indicate that dietary intakes of several essential minerals (e.g., calcium, copper, iron, magnesium, zinc) are less than recommended in many segments of the U.S. population and that mild-to-marginal deficiencies in these and other micronutrients are particularly likely in at-risk and underserved groups (e.g., women, children, elderly, minorities). Unfortunately, controlled studies of the relationships between micronutrients and chronic disease are few and the roles of potential mediating factors such as age, sex, body composition, special diets, lifestyle and genetic factors are poorly understood. For most micronutrients, the potential health benefits and mechanisms of action for physiological (healthy body weight and composition, energy metabolism, brain and cardiac function) and psychological (cognition, emotional and social adjustment, school/work performance) function have not been determined.
This project seeks to improve health and enhance quality of life by determining for healthy and at-risk groups micronutrient intakes that optimize physiological and psychological development, function and health. Specific project objectives are to: (1) develop new functional bases for establishing mineral element requirements; (2) identify mechanisms of action; and (3) determine the influence of mediating factors on mineral element requirements. This project is directly related to the following major components of the National Program Action Plan for Human Nutrition (107): Nutrient Requirements; Relationship between Diet, Genetics and Lifestyle and the Prevention of Chronic Disease; and Health-Promoting Interventions Strategies for Targeted Populations. The research addresses priority objectives including (1) determining functional markers of mineral intakes and status; (2) identifying mechanisms of action of mineral elements; (3) determining the influence of genetic, environmental and lifestyle factors on obesity and prevention of chronic disease; (5) identifying dietary intervention strategies effective with minority populations; (6) characterizing the role of mineral elements in achieving and maintaining optimal physiological and psychological development, function and health.
Controlled studies generate new knowledge to use in making recommendations for dietary intakes that promote optimal development, function and health throughout the life span. Dietary intakes and biochemical indices of mineral status are related to physiologic (e.g., healthy body weight and composition, physical fitness, energy metabolism, brain and cardiac function) and psychological (e.g., cognition, emotional and social adjustment, school/work performance) measures to determine importance of specific minerals for optimal function and development. A mobile field laboratory, community studies facilities, and a residential metabolic unit are used

to conduct survey, supplementation, fortification, and controlled feeding studies with healthy and at-risk groups (e.g., school-aged children, rural elderly, minorities). Randomized controlled trials evaluate the effects of feeding graded dose amounts of minerals, such as iron, zinc, copper and magnesium, in the context of mediating factors (e.g., genotype, controlled stressors). Animal and cell culture studies enhance the efficacy of human studies and help determine the mechanisms of action of functional outcomes.
This project provides experimentally-derived information needed to establish recommendations for dietary intakes of mineral elements throughout the life span and to help American consumers choose foods that optimize physical and mental performance, social and emotional adjustment, and prevent or ameliorate chronic diseases such as obesity, diabetes, depression and dementia. Resulting information is also useful for evaluating food assistance programs, special diets, the efficacy of taking dietary supplements, and the potential benefits of value-added foods to maintain health and well-being. Primary customers for the products of this research are agricultural and commodity groups, the food industry, supplement manufacturers, policy makers, health and nutrition professionals and the general public.

2. **List the milestones (indicators of progress) from your Project Plan.**

Year 1 (FY 2005)
• Plan, obtain institutional and school board approvals, and initiate studies for participation of children and adolescents to determine relationships among zinc and iron nutrition and cognitive function, psycho-educational performance, body composition and growth.
• Develop experimental protocol, initiate contacts with participating institutions, obtain institutional and administrative approvals, and initiate a cross-sectional epidemiologic study of nutrition, health and function in institutionalized and non-institutionalized elderly.
• Plan and initiate study of the effects of dietary zinc and copper on mechanisms of adaptation to endurance exercise training among in-bred strains of rats with different phenotypes for running capacity.
• Plan project and obtain approvals to initiate an observational study to determine the nutrient composition of foods in the food assistance programs and traditional foods on American Indian reservations.
• Complete study to determine whether dietary boron restriction in rats influences eye development and behavioral changes induced by feeding diets high in omega-3 fatty acids and to relate any influence to a biochemical mechanism.

Year 2 (FY 2006)
• Conduct study to determine in adolescents relationships between zinc and iron nutrition and cognitive function, psychoeducational performance, body composition, growth, and physical fitness at first site.
• Plan project and initiate study to determine magnesium requirements of postmenopausal women with outcomes of magnesium nutritional markers, inflammatory responses and cardiovascular risk factors.
• Conduct study of relationships among zinc, copper and magnesium nutrition and physical and mental health of healthy elderly.
• Analyze samples from study of effects of zinc and copper on mechanisms of adaptation to endurance exercise training among in-bred strains of rats with different phenotypes for running capacity; report results.
• Plan project and initiate study of effects of different body composition phenotypes of rats on copper and zinc metabolism with increased physical activity in rats.
• Complete the nutrient composition survey of components of food assistance program and traditional American Indian foods.

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• Plan and initiate study of interaction of dietary boron and essential fatty acids in rats.
• Establish communication, trust and collaborative partnerships with American Indian communities to begin discussions of health promotion, including obesity prevention.

Year 3 (FY 2007)
• Conduct study of zinc supplementation of adolescents at second site.
• Conduct study of magnesium requirements of postmenopausal women with outcomes of magnesium nutritional markers, inflammatory responses and cardiovascular risk factors.
• Plan and initiate study of copper on adaptation to increased physical activity of out-bred rats with different phenotypes for aerobic capacity.
• Develop nutrient database of commodity and traditional Native foods.
• Initiate community focus groups to identify and report perceived health concerns/needs of tribal communities.
• Analyze samples and data from observational study of the elderly; report results.
• Plan experimental protocol for study of effects of graded zinc intake on adaptation to increased physical activity in humans.

Year 4 (FY 2008)
• Conduct study of zinc supplementation of adolescents at third site.
• Complete study of magnesium needs of postmenopausal women; analyze samples and data, and report results.
• Develop experimental protocol, prepare and receive approval from host institution(s) and institutional review boards; initiate nutritional intervention study of the effects on mental and physical functions of the elderly.
• Apply results of formative evaluation to development of culturally-appropriate interventions approaches, materials and programs for health promotion and obesity prevention in American Indian communities.
• Complete the study of copper on adaptation to increased physical activity of out-bred rats with different phenotypes for aerobic capacity.

3a. List the milestones that were scheduled to be addressed in FY 2005. For each milestone, indicate the status: fully met, substantially met, or not met. If not met, why.

1. Plan, obtain institutional and school board approvals, and initiate studies for participation of children and adolescents to determine relationships among zinc and iron nutrition and cognitive function, psycho-educational performance, body composition and growth.

   Milestone Substantially Met

2. Develop experimental protocol, initiate contacts with participating institutions, obtain institutional and administrative approvals, and initiate a cross-sectional survey of nutrition, health and function in healthy elderly living in institutionalized and non-institutionalized environments.

   Milestone Substantially Met

3. Develop experimental protocol, conduct pilot study to verify that the breeds of rats respond as anticipated to copper and zinc restriction. Conduct training study.

   Milestone Substantially Met

4. Plan project and obtain approvals to initiate an observational study to determine the nutrient composition of foods in the food assistance programs and traditional foods on American Indian reservations.

   Milestone Fully Met

Project Number: 5450-51530-009-00D **Accession:** 0408299 **FY:** 2005

3a. 5. Complete a study in progress to determine if boron restriction in rats influences fatty acid composition of brain phospholipids, prostaglandin and catecholamine contents, and affect eye ultrastructure and brain function.

Milestone Fully Met

3b. List the milestones that you expect to address over the next 3 years (FY 2006, 2007, and 2008). What do you expect to accomplish, year by year, over the next 3 years under each milestone?

Year 2 (FY 2006)
• Conduct study to determine in adolescents relationships between zinc and iron nutrition and cognitive function, psychoeducational performance, body composition, growth, and physical fitness at first site in North Dakota, Texas or American Indian Reservations.
Anticipated outcome: establish relationships among indicators of zinc status and measures of physiological and biological functions in a group for whom no objective estimates of zinc intake are available.
• Plan project and initiate study to determine magnesium requirements of postmenopausal women with outcomes of magnesium nutritional markers, inflammatory responses and cardiovascular risk factors.
Anticipated outcomes: critically evaluate the recommendations for magnesium intake in a population group for whom no objectives are available, determine effects of magnesium intakes commonly consumed by older women on risk factors for cardiovascular disease and bone loss, and validate new measures of magnesium status.
• Conduct study of relationships among zinc, copper and magnesium nutrition and physical and mental health of healthy elderly.
Anticipated outcome: identify the prevalence of low nutritional status, particularly minerals, and its relationships with quality of life variables (e.g., psychological function and activities of daily living).
• Analyze samples from study of effects of copper on mechanisms of adaptation to endurance exercise training among in-bred strains of rats with different phenotypes for running capacity; report results.
Anticipated outcome: determine if physical activity level is a modifying factor in determining copper needs.
• Plan project and initiate study of effects of different body composition phenotypes of rats on copper and zinc metabolism with increased physical activity in rats.
Anticipated outcomes: determine if obesity, with and without exercise, is a moderating factor in copper metabolism.
• Complete the nutrient composition survey of components of food assistance program and traditional American Indian foods.
Anticipated outcome: provide a needed and practical nutrient data base for use by Native American health professionals to assist in planning healthful diets required in the promotion of healthy body weight.
• Plan and initiate study of interaction of dietary boron and essential fatty acids in rats.
Anticipated outcome: determine effects of omega 3 fatty acids on amelioration of adverse effects of restricted dietary boron on physiological and brain functions.
• Establish communication, trust and collaborative partnerships with American Indian communities to begin discussions of health promotion, including obesity prevention.
Anticipated outcome: develop a cooperative and culturally-appropriate program for healthy weight in a group in which obesity and diabetes are epidemic.

Year 3 (FY 2007)
• Conduct study of zinc supplementation of adolescents at second site.

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Anticipated outcome: determine the effects of zinc supplementation at recommended and higher amounts on growth, development, body composition, cognition, social adaptation, and physical fitness of youth.
• Conduct study of magnesium requirements of postmenopausal women with outcomes of magnesium nutritional markers, inflammatory responses and cardiovascular risk factors.
Anticipated outcomes: provide needed evidence for recommendation of magnesium intake in a population group for whom no objective are available, determine effects of magnesium intakes commonly consumed by older women on risk factors for cardiovascular disease and bone loss, and validate new measures of magnesium status.
• Plan and initiate study of copper on adaptation to increased physical activity of out-bred rats with different phenotypes for aerobic capacity.
Anticipated outcome: evaluate the effects of different levels of physical activity on copper nutritional status.
• Develop nutrient database of commodity and traditional Native foods.
Anticipated outcome: provide a needed and practical nutrient data base for use by Native American health professionals to assist in planning healthful diets required in the promotion of healthy body weight.
• Initiate community focus groups to identify and report perceived health concerns/needs of tribal communities.
Anticipated outcomes: develop a culturally-appropriate and sustainable program for health body weight in American Indian communities.
• Analyze samples and data from observational study of the elderly; report results.
Anticipated outcomes: determine effects of supplementation of limiting nutrients (e.g., zinc, magnesium, vitamin B12, etc.) on quality of life measures of the elderly.
• Plan experimental protocol for study of effects of graded zinc intake on adaptation to increased physical activity in humans.
Anticipated outcomes: determine if dietary zinc when fed in an amount consistent with usual intakes of US adults, compared to the recommended intake, results in impaired adaptation to increased physical activity at a level proposed in the Dietary Guidelines for Americans.
• Develop experimental protocol and conduct study in rats to evaluate the effect of moderate magnesium restriction on risk factors for metabolic syndrome (insulin resistance and hyperlipedemia); analyze results.
Anticipated result: determine the effects of magnesium intake, similar to that which occurs commonly in the American population, alters glucose and lipid metabolism and contributes to obesity.

Year 4 (FY 2008)
• Conduct study of zinc supplementation of adolescents at third site.
Anticipated result: determine the effects of zinc supplementation at recommended and higher amounts on growth, development, cognition, social adaptation, and fitness of youth; contribute information needed to develop zinc requirement of adolescents.
• Complete study of magnesium requirements for optimal cardiovascular, neurological and psychological function in postmenopausal women; analyze samples and data, and report results.
Anticipated outcome: provide evidence to Food and Nutrition Board for use in establishing recommendations for magnesium intakes for postmenopausal women to optimize health and valid biochemical indicators of human magnesium status for use in national nutritional surveys.
• Develop experimental protocol, prepare and receive approval from host institution(s) and institutional review boards; initiate nutritional intervention study of the effects on mental and physical functions of the elderly.
Anticipated outcomes: determine effects of supplementation of limiting nutrients

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(e.g., zinc, magnesium, vitamin B12, etc.) on quality of life measures of the elderly.
• Apply results of formative evaluation to development of culturally-appropriate interventions approaches, materials and programs for health promotion and obesity prevention in American Indian communities.
Anticipated outcomes: develop a culturally-appropriate and sustainable program for health body weight in American Indian communities.
• Complete exercise training study; analyze samples; report results.
Anticipated outcome: provide evidence of any effects of increased physical activity on copper nutritional status and physiological adaptations to aerobic exercise training.

4a. What was the single most significant accomplishment this past year?

Copper Requirement for Men
Controversy persists regarding the amount of copper needed to support health and optimal biological function. Because the recommendation for copper intake was empirically derived, we evaluated the effects of this recommended amount with a greater amount in men. We found increased losses of copper and increased energy needs during low-level physical activity in parallel with decreased activity of an energy-regulating copper-containing enzyme in muscle when 0.9 compared to 1.6 mg of copper was fed. These findings reveal that moderate copper intake adversely impacts the ability to perform work and relates this problem to the reduced activity of an important copper protein that regulates cellular energy production. This information will be useful to policy makers who make recommendations on nutrient intakes to promote health and well-being of the public.

4b. List other significant accomplishments, if any.

Beneficial effects of fish oil on vision most evident when boron status is low
The beneficial effects of fish oil, which is high in long chain omega-3 polyunsaturated fatty acids (PUFA), on vision was more marked in rats with a restricted intake of dietary boron, and the beneficial effects of boron on vision was more evident in rats fed a diet high in omega-6 PUFA. Some studies show that, compared to diets high in omega-6 PUFA, diets high in long chain omega-3 PUFA enhance sensory functions, cognitive functions and brain development, but other studies do not. The inconsistency may be the result of different intakes of another nutrient, such as boron, shown to affect similar functions as long chain omega-3 PUFA. The beneficial effects of fish oil or long-chain omega-3 PUFA supplementation may be most evident when dietary boron restriction, combined with high omega-6 PUFA intake, has impaired behavioral and vision functions.

4c. List any significant activities that support special target populations.

Scientists in the unit continue to work with American Indians to develop successful partnerships and to promote research on health promotion. An example is co-organization of the 3rd Annual American Indian Forum as part of the University of North Dakota's Indian Association Timeout Celebration, whose theme was "Enhancing the Health of Northern Plains Indians". More than a dozen speakers from throughout the region presented information on the current status of Native health in the Upper Midwest and research on physical and mental health, traditional diets and nutrition, and physical activity. Community efforts underway to promote improved health and major research needs were also identified and discussed. Products of the forum included improved communication and increased collaboration through networking of regional researchers and students interested in American Indian research, improved knowledge of ongoing AI research and barriers to future studies, and updating of the AIRO Directory and LSTSERV. This activity directly supports GFHNRC research to improve the nutrition and health of this at-risk and underserved population in our

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region, and facilitates accomplishment of the milestone related to health promotion and obesity prevention in American Indian communities.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**

The major accomplishment was the development of trust and respect among the American Indian tribes in North Dakota that resulted in the completion of survey of Native American health, nutrition and physical activity and preparation of a report summarizing the findings. The report has been distributed to participating Indian tribes and communities. This accomplishment falls within the USDA, ARS Strategic Plan to Improve the Nation's Nutrition and Health (Goal 4 of National Program 107 - Human Nutrition) and Objective 4.1.3: Determine food consumption patterns of Americans, including those of different ages, ethnicity, regions, and income levels. Provide sound scientific analyses of the U.S. food consumption information to enhance the effectiveness and management of the Nation's domestic food and nutrition assistance programs. It also addresses Action Plan component of Health Promoting Strategies for Targeted Populations and focuses on this at-risk and underserved population in our region; it also facilitates accomplishment of the milestone related to health promotion and obesity prevention in American Indian communities. A secondary accomplishment was the identification of the specificity of muscle cytochrome c oxidase activity as a marker on copper nutritional status and its potential for future research to determine the interaction of dietary copper restriction and supplementation on adaptation of muscle metabolism to increased physical activity. This work has lead to a study in men which confirmed that the activity of the enzyme is decreased when dietary copper is low and results in decreased copper nutritional status. This accomplishment falls within the ARS Strategic Plan to Improve the Nation's Nutrition and Health (Goal 4 of National Program 107 - Human Nutrition) and Objective 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients. It also addresses as the milestone (FY 2005) to relate dietary copper and physical activity.

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**

There are no CRADAs, licenses or patents to report.
Transfer of technical information to other scientists occurred through presentations at national and international meetings and professional publications. Knowledge about the health benefits of mineral nutrients was transferred by routine contacts to representatives of industry and policy-making and regulatory federal agencies. Transfer of knowledge to the public occurred through contacts with media representatives and by direct contacts with the public.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below).**

Popular Press
Lukaski, H.C. Coalition Provides Wealth of Health Advice. Grand Forks Herald, July, 2005.

Nielsen, F.H. Don't Overlook Benefits of Diets that Contain Fish. Grand Forks Herald. March 2005.

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Penland, J.G. Cold Weather and Nutrition Go Hand in Hand. Grand Forks Herald. February 2005.

Media Coverage
H. Lukaski summarized research findings that were described in stories on zinc, health and performance by Reuters news service, Women's Health and Self.

J. Penland was cited in numerous stories on minerals (boron, calcium, copper, magnesium and selenium) and sleep, brain function and menstrual symptomology, appearing in magazines such as Organic Style, First for Women, and Natural Health. In addition, the media extensively covered his work on zinc and cognitive function in young adolescents. He conducted interviews with representatives of more than 20 media outlets, including Associated Press, Reuters News Service, Bloomberg News, New York Science News, Los Angeles Times, Reader's Digest, ABC and BBC, and this work was reported by more than 30 additional wire service, television, radio, newspaper, magazine and internet outlets.

Presentations
We made 23 presentations to industry, governmental and educational entities, 8 of which occurred during the 4th Quarter of FY-04, and 15 during the 1st-3rd Quarters of FY-05. The most important presentations included:

Lukaski, H.C. and Penland, J.G. Diet, Physical Activity and Health Among Northern Plains Indians. American Indian Living Radio Broadcast, September 28, 2004.

Lukaski, H.C. Zinc, Magnesium and Copper Requirements and Exercise. National Academy of Sciences, Institute of Medicine, Committee on Military Nutrition Research, Workshop on Mineral Requirements for Cognitive and Physical Performance of Military Personnel, Washington, DC, June 15, 2005.

Nielsen, F.H. Magnesium Balance as an Indicator of Magnesium Status and Experimental Magnesium Depletion in Humans. Gordon Research Conference on Magnesium in Biochemical Processes and Medicine, Ventura, CA, February 10, 2005.

Penland, J.G. Zinc and Other Mineral Nutrients Related to Cognition and Behavior. National Academy of Sciences, Institute of Medicine, Committee on Military Nutrition Research, Workshop on Mineral Requirements for Cognitive and Physical Performance of Military Personnel, Washington, DC, June 15, 2005.

Scientific Publications: **Log 115:**

1. Clodfelder, B.J., Gullick, B.M., Lukaski, H.C., Neggers, Y., Vincent, J.B. 2005. Oral administration of the biomimetic [Cr3O(O2CCH2CH3)6(H2O)3]+ increases insulin sensitivity and improves blood plasma variables in healthy and type 2 diabetic rats. Journal of Biological Inorganic Chemistry. 10:119-130. **0000180162**
2. Johnson, W.T., Johnson, L.K., Lukaski, H.C. 2005. Serum superoxide dismutase 3 (extracellular superoxide dismutase) activity is a sensitive indicator of Cu status in rats. Journal of Nutritional Biochemistry. 16:682-692. **0000173651**
3. Kyle, U.G., Genton, L., Lukaski, H.C., Dupertuis, Y.M., Slosman, D.O., Hans, D., Pichard, C. 2005. Comparison of fat-free mass and body fat in Swiss and American adults. Nutrition 21:161-169. **0000180960**
4. Lukaski, H.C. 2005. Assessing Muscle Mass. In: Heymsfield, S.B., Lohman, T.G., Wang, Z.M., Going, S.B. (Eds.) Human Body Composition. Second Edition. Champaign, IL, Human Kinetics. p. 203-218. **0000143107**
5. Lukaski, H.C. 2005. Low dietary zinc decreases erythrocyte carbonic anhydrase **0000159796**

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activities and impairs cardiorespiratory function in men during exercise. American Journal of Clinical Nutrition. 81:1045-1051.

6. Lukaski, H.C., Johnson, P.E. 2005. Dietary copper (Cu) at the recommended intake decreases muscle cytochrome c oxidase (CCO) activity and alters metabolic responses during exercise in men [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A982. **0000170041**

7. Lukaski, H.C., Penland, J.G. 2005. Zinc, Magnesium, Copper, Iron, Selenium, and Calcium in Assault Rations: Roles in Promotion of Physical and Mental Performance. In: Oria, M.P., Erdman, J.W., (Editors) Nutrient Composition of Rations for Short-Term, High Intensity Combat Operations. Washington, D.C.: National Academy Press. p. 223-235 **0000169545**

8. Moulton, P.L., Petros, T.V., Apostal, K.J., Park, R.V., II, Ronning, E.A., King, B.M., Penland, J.G. 2005. Alcohol-induced impairment and enhancement of memory: A test of the interference theory. Physiology and Behavior. 85(3):240-245. **0000163732**

9. Penland, J.G., Lukaski, H.C., Gray, J.S. 2005. Zinc affects cognition and psychosocial function of middle-school children [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A973. **0000171893**

10. Schoeller, D.A., Tylavsky, F., Baer, D.J., Borrud, L., Chumlea, W.C., Earthman, C., Fuerst, T., Harris, T., Heymsfield, S., Horlick, M., Lukaski, H.C. 2005. Fan beam dual x-ray absorptiometry underestimates fatness in adults. American Journal of Clinical Nutrition. **0000158003**

Approved: CHANDLER LAURENCE D **Date:** 09/20/2005

Project Number: 5450-51530-009-01T **Accession:** 0406520 **FY:** 2005
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH

Start Date: 10/01/2002 **Term Date:** 09/30/2007

National Programs: 107 N Human Nutrition

Title: DETERMINATION OF THE EFFECTS OF BUCKWHEAT ON MANAGEMENT OF PRE-DIABETES AND NON-INSULIN DEPENDENT DIABETES (NIDDM) IN ANIMAL MODELS

Period Covered From: 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-3-0402

Organization Name: MINN-DAK GROWERS, LTD.

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

None.

4b. List other significant accomplishments, if any.

None.

4c. List any significant activities that support special target populations.

None.

4d. Progress report.

This report serves to document research conducted under trust agreement #58-5450-3-0402 between ARS and Minn-Dak Growers, Ltd. Additional information can be found in the report for the parent project 5450-51530-009-00D Micronutrient roles in physiology and health.

Male, Zucker obese (ob/ob) rats were matched by body weight, randomized by group and fed a purified diet (AIN-93) supplemented with a standard dose of fagopyritols (5 and 15 mg/kg body weight) isolated from raw farinetta during a 12-week period. After six and 12 weeks of feeding the diets, the rats were fasted and administered a standardized oral glucose tolerance test (75 g glucose per os) with blood obtained for determination of glucose and insulin. There was no effect of diet on glucose or insulin responses after six weeks of dietary treatment. At the end of 12 weeks of feeding, rats fed the higher dose of fagopyritols had a significant decrease in the area under the glucose curve (20%) during the oral glucose tolerance test compared the rats consuming the control and lower fagopyritol diets. Diet composition did not affect the area under the insulin curve during the glucose challenge tests. These findings indicate a beneficial effect of fagopyritols added to the diet on apparent glucose handling (utilization) in an animal model of obesity and pre-diabetes. They provide limited support for continued research to identify health-promoting effects of buckwheat in obesity and diabetes.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

See 4D above.

Project Number: 5450-51530-009-01T **Accession:** 0406520 **FY:** 2005

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**

FY 2006 - Studies will be initiated to determine the effects of isolated fagopyritols on the prevention of insulin resistance, a precursor of type 2 diabetes. One experiment will utilize in-bred rats with a phenotype to develop insulin resistance. The animals will be fed graded doses of fagopyritols from food (buckwheat) and extracts added to the diet on oral glucose tolerance and insulin sensitivity. Potential mechanisms of action will investigated including glucose transporters in peripheral tissues, insulin signaling systems and insulin secretion rates.

FY 2007 - If the proposed rodent studies reveal that buckwheat has beneficial effects on glucose and insulin metabolism, planning for a study of buckwheat in amelioration of pre-diabetes in humans with symptoms of the metabolic syndrome will be planned and initiated.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below).**

None.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/23/2005

Project Number: 5450-51530-009-02N **Accession:** 0408500 **FY:** 2005
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 10/18/2004 **Term Date:** 08/31/2006

National Programs: 107 N Human Nutrition

Title: NUTRITION EFFECTS ON COGNITIVE PERFORMANCE OF YOUNG VERSUS ELDERLY COMMUNITY-LIVING ADULTS

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-5-0101N

Organization Name: UNIV OF NORTH DAKOTA

Progress and Outcomes:

4d. **Progress report.**

This report serves to document research conducted under non-funded cooperative agreement #58-5450-5-101N between ARS and University of North Dakota Psychology Department. Additional information can be found in the report for the parent project 5450-51530-009-00D, Micronutrient roles in physiology and health.

To date, data have been collected from 30 younger (21-35 y) and 13 older (51-75 y) healthy community-dwelling adults in a study designed to determine whether dietary intakes and status of essential mineral nutrients, particularly magnesium and zinc, are mediating factors in the memory performance of older and younger adults. Findings will help elucidate the role of mineral nutrition in memory function and in age-related changes in memory performance.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/23/2005

Project Number: 5450-51530-009-03N **Accession:** 0409328 **FY:** 2005
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: DAVID M KLURFELD
Start Date: 05/26/2005 **Term Date:** 03/31/2006

National Programs: 107 N Human Nutrition

Title: ASSESSMENT OF MINERAL LOSSES IN SWEAT DURING PHYSICAL ACTIVITY

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-5-0107N
Organization Name: GATORADE SPORTS SCIENCE INSTIT.

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?

2. List the milestones (indicators of progress) from your Project Plan.

4a. What was the single most significant accomplishment this past year?

None.

4b. List other significant accomplishments, if any.

None.

4c. List any significant activities that support special target populations.

None.

4d. Progress report.

This report serves to document research conducted under non-funded cooperative agreement #58-5450-5-107N between ARS and Gatorade Sport Science Institute. Additional information can be found in the report for the parent project 5450-51530-009-00D Micronutrient roles in physiology and health.

A study to determine the effects of physical training in elite male and female athletes was planned and is in progress. Sweat samples will be collected from various regions of the body during intense physical activity. The samples will be prepared by the research collaborators and delivered to the Center for analyses of mineral element concentrations.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/23/2005

MICRONUTRIENT ABSORPTION AND METABOLISM

MANAGEMENT UNIT

5450-020-00

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: MARY J KRETSCH
Start Date: 01/15/2004 **Term Date:** 01/14/2009
National Programs: 107 N Human Nutrition
Title: MINERAL UTILIZATION AND BIOAVAILABILITY IN THE 21ST CENTURY, WITH CHANGING DIETS AND AGRICULTURAL PRACTICES
Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?**

This is the annual report for the OSQR-approved project, 5450-51000-035-00D, "Mineral Utilization and Bioavailability in the 21st Century, with Changing Diets and Agricultural Practices".
Current trends and proposed changes in the US diet can substantially influence the mineral nutrient status of the population. Such changes include: a) an emphasis on plant-based diets with limited intake of red meat, b) mineral fortification or supplementation of diets proposed by health care professionals, or independently initiated by producers, and c) new technologies to produce genetically- or chemically- modified foods, or to stabilize and enhance the bioavailability of nutrients added to foods. The changes that may affect the mineral contents and/or the absorption and utilization (bioavailability) of dietary minerals, or disrupt the balance among interacting dietary minerals must be identified. Effective approaches such as dietary modifications, fortification/supplementation strategies, and agricultural and food production approaches must be identified to enhance human mineral nutrition and promote health.
The project has 3 objectives: (1) Determine how shifts in agricultural and dietary practices, such as the availability of functional/genetically modified foods and emphasis on plant-based diets with reductions in meat consumption will impact the intake, bioavailability, and dietary requirements of minerals. (2) Determine the effectiveness of current and proposed mineral fortification/supplementation practices for improving mineral nutrition while avoiding excessive or imbalanced mineral intakes. (3) Determine the mechanisms of uptake, transport, and retention of food minerals and how mineral nutritional status influences these mechanisms to impact the bioavailability of essential minerals, non-nutritive metals, and other food components.
This project will evaluate modifications that can enhance trace element nutrition, with emphasis on selenium (Se), iron (Fe), zinc (Zn), and copper (Cu). Agricultural practices that can influence the mineral contents of foods, especially Se, will be evaluated, with assessment of the health properties of such Se-enhanced foods. The influence of reduced dietary Fe and Zn bioavailability will be evaluated in human studies that will help define quantitative mineral needs, elucidate the effect of dietary phytate in combination with dietary Ca fortification, and evaluate the effectiveness of different forms of iron used for food fortification. An algorithm will be developed to assess the Zn bioavailability of diets from generally available food composition data. The basic mechanisms of uptake, transport, and retention of food minerals will be evaluated to determine how mineral nutritional status and intake impact the absorption and metabolism of essential minerals, in interaction with non-nutritive metals and food components.

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This research is directly related to three components of the ARS National Human Nutrition 107 research plan: (1) Human Nutrition Requirements, (6) Health Promoting Properties of Plant and Animal Foods, and (7) Bioavailability of Nutrients and Food Components.
This research will provide critical information about the biological control of mineral absorption, of nutritionally important mineral-mineral interactions, and of bioavailability of minerals from various food and fortification sources. This knowledge is essential for the development of Dietary Reference Intakes, FDA food and dietary supplement regulations, and other U.S. nutrition and food policy guidelines for providing safe and adequate mineral nutrition from the food supply, and will be useful for the agricultural and food production sectors in the development of healthful foods.
The results of these studies will be useful to food producers for the development and promotion of healthy foods, and to health care officials and educators for the development of dietary advice that contributes to optimal nutrition. The ultimate beneficiary is the American consumer through policies and guidelines set by scientists and health care professionals based on these research findings and through transfer of enhanced agricultural products that improve nutrition worldwide.

2. List the milestones (indicators of progress) from your Project Plan.

(This CRIS project began in January, 2004, and has now been in effect for 1.5 years)

Year 1 (Addressed in FY2005)
Complete 1st yr of wheat & buckwheat Se accumulation studies (1.1)
Complete transgenic wheat accumulation of Se study (1.1)
Develop cells lines and constructs for antioxidants & gene expression study (1.5)
Develop Se speciation methodology (1.1)
Complete interim blood analyses for Fe excretion study & write initial paper (1.9)
Enroll subjects in elemental Fe powder study (2.1)
Complete antibody prep & perform studies on up/down regulation of mineral transport in Caco-2 cells (3.1).
Complete studies on Cu transporter trafficking in Caco-2 cells given high Zn media (3.2).

Year 2 (Addressed in FY2006)
Complete 2nd yr of wheat & buckwheat Se accumulation studies (1.1)
Complete anti-oxidants & gene expression study (1.5)
Complete 1st yr of organic/conventional foods study (1.1)
Report transgenic wheat study (1.1)
Complete Se speciation in wheat study (1.1)
Develop Zn algorithm & prepare paper (1.7)
Enroll subjects in Zn requirement study (1.6)
Complete elemental Fe powders study (2.1)
Wrap up studies on up/down regulation of mineral transport in Caco-2 cells. Write manuscripts (3.1)
Wrap up studies on Cu transporter trafficking. Write manuscripts (3.2)

Year 3 (Addressed in FY2007)
Complete feeding portion of human high-Se beef study (1.3)
Report antioxidants & gene expression study (1.5)
Report wheat & buckwheat accumulation studies (1.1)
Complete 2nd yr of organic/conventional foods study (1.1)
Complete high-Se beef and aberrant crypt study (1.3)
Complete Zn requirement study (1.6)

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Report elemental Fe powders study (2.1)
Complete studies on low mineral intakes, metal transporters, and cadmium accumulation in intestinal cells (3.3)
Complete studies on relationship between Cu deprivation & Hephestin activity (3.4)

Year 4 (Addressed in FY2008)
Complete analyses for human high-Se beef study (1.4)
Complete differences in bioavailability of mineral nutrients from organic/conventional broccoli (1.2)
Report high-Se beef and aberrant crypt study (1.3)
Complete study of phytate X Ca & Zn bioavailability (1.8)
Report Zn requirement study (1.6)
Complete microencapsulated Fe study (2.2)
Wrap up Cu/Heph/Fe absorption studies. Write manuscripts (3.4)
Complete study on marginal mineral & Cd exposure, analyze data (3.5).

Year 5 (Addressed in FY2009)
Report human high-Se beef study (1.4)
Complete comparison of high-Se foods aberrant crypt study (1.2)
Report comparison of high-Se foods and aberrant crypt study (1.3)
Report organic & conventional foods study (1.1)
Report study of phytate X Ca & Zn bioavailability (1.8)
Complete final report of Fe excretion data (1.9)
Report microencapsulated Fe study (2.2)
Wrap up studies in objective 3.5. Write manuscripts.
Complete new 5-year proposal.

3a. List the milestones that were scheduled to be addressed in FY 2005. For each milestone, indicate the status: fully met, substantially met, or not met. If not met, why.

1. Complete 1st yr of wheat & buckwheat Se accumulation studies (1.1)
   Milestone Fully Met

2. Complete transgenic wheat accumulation of Se study (1.1)
   Milestone Fully Met

3. Develop cells lines and constructs for antioxidants & gene expression study (1.5)
   Milestone Substantially Met

4. Develop Se speciation methodology (1.1)
   Milestone Fully Met

5. Complete interim blood analyses for Fe excretion study & write initial paper (1.9)
   Milestone Substantially Met

6. Enroll subjects in elemental Fe powder study (2.1)
   Milestone Fully Met

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3a. 7. Complete antibody prep & perform studies on up/down regulation of mineral transport in Caco-2 cells (3.1)

Milestone Fully Met

8. Complete studies on Cu transporter trafficking in Caco-2 cells given high Zn media (3.2)

Milestone Fully Met

3b. List the milestones that you expect to address over the next 3 years (FY 2006, 2007, and 2008). What do you expect to accomplish, year by year, over the next 3 years under each milestone?

Year 2 (Addressed in FY2006)
- Complete 2nd yr of wheat & buckwheat Se accumulation studies (1.1) This study will provide practical information concerning the ability of these foods to be used as supplemental sources of dietary Se. Foods enriched in selenium are in demand by consumers, thus selenium-enriched wheat and buckwheat could represent a 'value-added' agricultural product.
- Complete anti-oxidants & gene expression study (1.5) This study will determine whether dietary selenium and antioxidants interact to affects gene expression.
- Complete 1st yr of organic/conventional foods study (1.1) Organic foods are often advertised as being more nutritious than comparable conventionally-grown counterparts, however very few controlled studies have examined this. This comprehensive study will compare the nutritional value of broccoli grown in California by certified organic techniques or by conventional horticulture.
- Report transgenic wheat study (1.1) Publication of this study will provide information regarding whether the chemical form of selenium in wheat can be changed by transgenic techniques.
- Complete Se speciation in wheat study (1.1) Determining the chemical form of Se in wheat is critical to determining the biological activity of such selenium.
- Develop Zn algorithm & prepare paper (1.7) Publication of this paper will provide a useful estimation of zinc bioavailability of diets, facilitating dietary evaluation and planning to improve zinc nutrition.
- Enroll subjects in Zn requirement study (1.6). This study, when completed, will provide new information on the relationship between zinc intake, absorption, and absorptive adaptation to meet zinc requirements.
- Complete elemental Fe powders study (2.1). This study method is designed to evaluate the bioavailability of elemental iron powders, and will also provide unique data on the relationship of nonheme iron absorption in healthy women with serum pro-hepcidin and ferritin.
- Wrap up studies on up/down regulation of mineral transport in Caco-2 cells. Write manuscripts (3.1). The outcome of this study should provide new information about the effects of supra-nutritional amounts of various minerals on the regulatory mechanisms of mineral absorption, which will help set guidelines for safe and adequate intakes of dietary minerals.
- Wrap up studies on Cu transporter trafficking. Write manuscripts (3.2). The outcome of this study should provide basic information about the effect of supra-nutritional amounts of dietary zinc on the control of copper absorption and regulation, and provide useful information for setting policy on dietary zinc allowances.

Year 3 (Addressed in FY2007)
- Complete feeding portion of human high-Se beef study (1.3) High-selenium beef may be an excellent food for providing supplemental dietary selenium; a human feeding study will provide information concerning the bioavailability and potential health

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benefits of selenium from high-selenium beef.
- Report antioxidants & gene expression study (1.5) This publication will detail the results of a series of studies examining how dietary ingredients affect activity of antioxidant genes and modulate overall oxidative status of the cell.
- Report wheat & buckwheat accumulation studies (1.1) This publication will report the results of all studies examining selenium accumulation in wheat and buckwheat and the bioavailability of selenium from those sources.
- Complete 2nd yr of organic/conventional foods study (1.1) This multi-year study will provide a robust data set that compares the nutritional profile of broccoli grown conventionally or by organic techniques and will help answer the question of whether organically grown vegetables have superior nutritional characteristics.
- Complete high-Se beef and aberrant crypt study (1.3) This study will provide information as to whether selenium from beef is effective for reducing the risk of colon cancer.
- Complete Zn requirement study (1.6) This study will provide new information on the relationship between zinc intake, absorption, and absorptive adaptation to meet zinc requirements.
- Report elemental Fe powders study (2.1) Publication will provide unique data on the relationship of nonheme iron absorption in healthy women with serum pro-hepcidin and ferritin.
- Complete studies on low mineral intakes, metal transporters, and cadmium accumulation in intestinal cells (3.3). The outcome of this study should provide basic information about mechanisms of marginal nutritional deficiencies such as zinc, iron, and calcium on the regulation of absorption of the toxic element cadmium, and provide useful information for setting policy on dietary allowances for cadmium exposure to humans.
- Complete studies on relationship between Cu deprivation & hephaestin activity (3.4). Completion of this study will provide valuable basic scientific information about dietary nutrient regulation of an important intestinal copper-dependent ferroxidase required for the efficient absorption of dietary iron.

Year 4 (Addressed in FY2008)
- Complete analyses for human high-Se beef study (1.4) This study will provide information concerning the bioavailability and potential health benefits of selenium from high-selenium beef.
- Complete differences in bioavailability of mineral nutrients from organic/conventional broccoli (1.2) This study will help answer the question of whether organically grown vegetables have superior nutritional characteristics.
- Report high-Se beef and aberrant crypt study (1.3) This study will provide information as to whether selenium from beef is effective for reducing the risk of colon cancer.
- Complete study of phytate X Ca & Zn bioavailability (1.8) This study will test the impact of a phytate X Ca X Zn interaction in practical human diets, and may be related to current recommendations to increase intake of whole grains and dairy products while reducing meat products.
- Report Zn requirement study (1.6) Publication will provide new information on the relationship between zinc intake, absorption, and absorptive adaptation to meet zinc requirements.
- Complete microencapsulated Fe study (2.2) Because the methodology for this study was tested and found to be ineffective with elemental iron powders, this planned milestone will be replaced with a milestone to "Complete validation of Caco-2 cell results with human absorption results on iron bioavailability from agricultural products."
- Wrap up Cu/Heph/Fe absorption studies. Write manuscripts (3.4) Completion of this study will provide valuable basic scientific information about dietary nutrient

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regulation of an important intestinal copper-dependent ferroxidase required for the efficient absorption of dietary iron.
- Complete study on marginal mineral & Cd exposure, analyze data (3.5). Completion of this study will give insight into the mechanisms whereby mineral nutrient status of the mother can influence the transfer of the toxic element cadmium to the fetus.

4a. What was the single most significant accomplishment this past year?

a. Developed a mathematical algorithm to estimate dietary zinc absorption from food composition data. To make practical use of sensitive and resource-intensive measurements of zinc absorption from human diets, the data should be applied to enable accurate predictions based on less intensive food composition measurements. Recent data from this research center, which nearly doubled the available data on zinc absorption from human diets, was applied to predict zinc absorption from food composition by regression analysis. The resulting algorithms explained ~83% of the variability in zinc absorption based on the dietary content of zinc, phytic acid, calcium and iron. Such readily available estimates of zinc absorption from diets are useful to evaluate and improve the diets of populations at risk of zinc deficiency, especially children in developing countries, and to evaluate the potential impact of changes in US diets.

4b. List other significant accomplishments, if any.

b. Demonstrated that impaired iron absorption in copper deficient animals is associated with a reduction in the copper-containing hephaestin protein. Adequate copper nutriture is required for efficient iron absorption. Iron release from the absorptive cells of the gut into the blood requires the ferroxidase hephaestin to convert iron-2 to iron-3 oxidation state, and copper is required for the activity and stability of this enzyme. It was shown in a series of experiments that copper deficiency in weanling rats reduced iron absorption and produced iron deficiency anemia. In addition, it was shown by Western blotting that copper deficiency reduced the amount of this ferroxidase protein, and upon refeeding copper to copper-deficient rats, the ferroxidase protein was restored. Further understanding of the role of copper in iron absorption will be useful in resolving the problems of nutritional anemias that are not responsive to supplemental iron.

c. Showed that copper deficiency impairs red blood cell formation in addition to impairing iron absorption. In most mammalian species, dietary copper deficiency leads to iron deficiency anemia. One of the reasons for this phenomenon is reduced iron absorption from the gut caused by reduced protein concentration of a copper-dependent iron ferroxidase protein in the intestinal absorptive cells. However, copper deficiency is known to affect blood cell formation in pigs and humans. A recent study in our laboratory showed that injections of iron into copper-deficient, anemic rats did not raise serum iron concentration and did not cure the anemia; however, liver iron was elevated 3-fold. These data suggest that copper is required for red cell formation and/or hemoglobin synthesis. An enhanced understanding of the role of copper in blood cell formation and hemoglobin synthesis will help set recommendations for dietary copper intakes.

d. Determined that excess cadmium retention in the intestines of animals marginally deficient in some minerals is independent of metallothionein protein. Marginal dietary deficiencies of zinc, iron, and calcium in mammals greatly enhance the absorption of the toxic element cadmium. With these deficiencies, cadmium fed at very low dietary levels accumulates in the intestinal absorptive cells, kidney, and liver. Intestinal metallothionein is a natural compound induced by high intakes of cadmium to detoxify the element. We found that accumulation of cadmium in the

intestinal cells of these deficient animals was not associated with elevated metallothionein. In addition, mice genetically altered to be free of functional metallothionein and made marginally deficient in zinc, iron, and calcium, were found to have elevated intestinal cadmium. These data suggest that metallothionein is not induced or required to accumulate cadmium in the intestine when the metal is fed at low levels to animals marginally deficient in zinc, iron, and calcium, and that cadmium accumulation occurs by some other unknown mechanism.

e. Delineated mechanisms of antioxidant activity of the sulforaphane and selenium chemicals found in plant foods (especially cruciferous vegetables). A series of experiments established models and conditions for studying how sulforaphane and selenium affect oxidative stress and modulate expression of antioxidant genes. Molecular and chemical methods were used to demonstrate that thioredoxin reductase is regulated by multiple dietary components, especially selenium and sulforaphane, and that silencing thioredoxin reductase results in greatly increased oxidative stress. An improved understanding of how diet influences oxidative stress will help determine dietary recommendations that can reduce the risk of chronic diseases such as cancer.

f. Determined that selenium-enhancement of buckwheat reduced selenium bioavailability. Plants grown on soils high in selenium accumulate selenium in the edible tissue, and such foods have the potential to be a good dietary source of the nutrient. A series of studies have demonstrated that the edible flour of wheat and buckwheat grown on high-selenium soils will accumulate selenium as much as 20-times beyond the average. Human and animal studies have demonstrated that selenium from high-selenium processed wheat is bioavailable and able to induce selenoprotein production and replete tissue stores of selenium. Buckwheat selenium, however, was not as bioavailable to rodents as pure chemical sources of selenium. These studies demonstrate that wheat has potential to be a good source of supplemental selenium, but the low bioavailability of buckwheat selenium may decrease its potential value as a supplemental source of selenium. The selenium enhancement of grains would be useful for increasing dietary selenium intakes, with possible health benefits related to the risk of chronic diseases.

g. Determined that serum pro-hepcidin was not significantly correlated with iron absorption in premenopausal women. Comparisons with human iron absorption measurements can contribute to understanding the significance of hepcidin, a recently discovered peptide with antimicrobial properties, which is proposed to play a central role in the biological regulation of iron absorption. Serum pro-hepcidin concentrations were relatively stable in women assessed 16 weeks apart, and correlated directly with serum ferritin, an indicator of body iron stores. However, in contrast with serum ferritin, pro-hepcidin concentrations were unrelated to iron absorption in 28 healthy women. A better understanding of the biological control of iron absorption will help in developing recommendations for iron intake to prevent or treat iron disorders associated with altered absorption, especially those commonly associated with infection or inflammatory diseases.

h. Sensitively assessed iron excretion in women as well as men. For several decades, dietary recommendations for iron intake have relied substantially on a single study of iron excretion, sensitively measured by isotope dilution in men only nearly 50 years ago. An update of this work confirmed the iron excretion measurements in healthy men, and for the first time provided such measurements in women. The data suggested that iron excretion is unrelated to body iron status in men, but that it determines the iron status of pre-menopausal women, because of the substantial influence of menstrual iron losses. These are the first such iron

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excretion data from women, and will contribute to the data available for setting dietary recommendations for iron intake as affected by gender.

4c. List any significant activities that support special target populations.

None

4d. Progress report.

None

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

This research is directly related to three components of the ARS National Human Nutrition 107 research plan: (1) Human Nutrition Requirements, (6) Health Promoting Properties of Plant and Animal Foods, and (7) Bioavailability of Nutrients and Food Components. The accomplishments below directly contribute to the accomplishment of ARS Strategic Plan Goal #4, Improve the Nation's Nutrition and Health.

- Incorporated a selenium accumulation gene into wheat, and tested the effects of various growing conditions to increase the uptake of selenium by the transfected cultivars. (In collaboration with investigators at USDA/ARS, Albany, CA, and USDA/ARS, Houston, TX.) Development of selenium accumulating strain of wheat may someday allow production of wheat with extremely high concentrations of selenium which could be used as a source of supplemental Se or as a selenium-fortificant in cereal grain-based products.

- Developed methods to determine the chemical form of the selenium in foods, especially selenium in methylated forms that may be especially anticarcinogenic. The selenium compounds in dried vegetable powder were extracted and analyzed by HPLC coupled to inductively coupled plasma mass spectrometry. Further development of this methodology will allow us to better predict the bio-activity of selenium from numerous common plant foods.

- Demonstrated the potential antioxidant properties of selenium-enriched broccoli, which in rat diets was associated with decreased risk of some cancers. Extracts of selenium-enriched broccoli or of broccoli rich in sulforaphane effectively reduced DNA strand breaks in cultured liver cells of rats. Because DNA strand breaks are a major initiating event of many cancers, these results suggest that some of the cancer protective effects of sulforaphane and selenium may be protection against oxidative stress.

- Discovered a novel role for selenium in the up-regulation of cell cycle related genes, that may lead to a better understanding of the essentiality of selenium as a nutrient and its involvement in cancer prevention.

- Demonstrated that, compared to a conventional farming technique, an organic farming method had limited influence on several nutritional characteristics of broccoli, including trace minerals, multiple individual glucosinolates, primary glucosinolate breakdown products, vitamin C and phenolic acids.

- Discovered that enhancing the selenium content of broccoli decreased total glucosinolate content, specifically sulforaphane, and changed the phenolic profile, especially reducing the content of hydroxy-cinnamic acids, suggesting that it may not be possible to simultaneously maximize all bioactive ingredients in a food, as enrichment with one compound may cause a concomitant decrease in another.

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- Determined that zinc interference with copper transport appears unrelated to the expression of the human copper transporter, ATP7b. An understanding of the interaction between copper and zinc absorption will be useful in setting recommendations for balanced dietary intakes of the two elements.

- Confirmed that copper deficiency reduces iron absorption in rats of both sexes, resolving some mixed results from other laboratories. An understanding of the role of copper in iron absorption will be useful in setting recommendations for balanced dietary intakes and addressing the causes of nutritional anemias.

- Discovered that marginal intakes of zinc, iron, and calcium greatly enhanced the accumulation of cadmium in the upper small intestine, leading to a higher accumulation of the toxic metal in the liver and kidneys, which suggests that populations with these nutrient deficiencies are especially susceptible to cadmium toxicity.

- Demonstrated that the 10% of people of Northern European origin who inherited the hemochromatosis mutation from one parent (heterozygous carriers) absorbed both heme and nonheme iron similar to those without the mutation, even from a meal highly fortified with iron and vitamin C, suggesting that current food iron fortification policies do not place this large group at increased health risk.

- Demonstrated that elemental iron powders commonly used to fortify staple foods with iron were less bioavailable to rats than iron from ferrous sulfate, and commercial versions differed considerably, suggesting that higher concentrations of these forms may be needed if they are used in international iron fortification programs.

- Showed that reduced and electrolytic iron sources were approximately 50 and 85% as effective as ferrous sulfate and 5 mg iron in the heme form was half as effective as 50 mg of iron from ferrous sulfate for improving body iron in premenopausal women. This research with humans will help to determine the most useful forms of iron to use in supplementation and fortification programs to reduced iron deficiency anemia worldwide.

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**

JR Hunt served as reviewer for the Food and Nutrition Board, Institute of Medicine, National Academy of Sciences report on "Nutrient Composition of Rations for Short-term, High-Intensity Combat Operations", Washington DC, National Academies Press, 2005.

JR Hunt began service as member of the National Academies, Institute of Medicine, Food and Nutrition Board Committee on Mineral Requirements for Cognitive and Physical Performance of Military Personnel, April 2005-February, 2006.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below).**

(The following includes items from the 4th Quarter of FY-04.) Our research on the genetics of iron absorption was favorably featured in an editorial in the American

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Journal of Clinical Nutrition, and in an article in Agricultural Research. In addition to presentations with the abstracts below, four presentations were given on iron and zinc absorption at a Workshop on Mineral Requirements for Cognitive and Physical Performance of Military Personnel, Food and Nutrition Board, Institute of Medicine in Washington, DC, to the Danish Nutrition Society, in Frederiksberg, Denmark, to a HarvestPlus workshop in Norwich, UK, and to the American Society for Clinical Laboratory Sciences-North Dakota Chapter. Four nutrition articles were written for the Grand Forks Herald.

Scientific Publications: **Log 115:**

1. Hunt, J.R., Zeng, H. 2004. Iron absorption by heterozygous carriers of the HFE C282Y mutation associated with hemochromatosis. American Journal of Clinical Nutrition. 80:924-31. **0000157457**
2. Miller, K.B., Newman Jr, S.M., Caton, J.S., Finley, J.W. 2004. Manganese alters mitochondrial integrity in the hearts of swine marginally deficient in magnesium. Biofactors. 20:85-96. **0000150852**
3. Hunt, J.R. 2005. Letter to the editor: absorption of iron from ferritin. American Journal of Clinical Nutrition. 81(5):1178-79. **0000173235**
4. Combs, G.F., Jr. 2005. Geological Impacts on Nutrition (Chapter 7). In: Stone, D. Essentials of Medical Geology. Elsevier Publishers, Sweden. pp 161-177. **0000167097**
5. Hunt, J.R. 2005. Iron: physiology, requirements, and dietary sources. In: Cabellero, B., Allen, L. and Prentice, A., editors. Encyclopedia of Human Nutrition, 2nd Ed. Elsevier Limited. p. 82-90. **0000172057**
6. Reeves, P.G., Chaney, R.L., Simmons, R.W., Cherian, M.G. 2005. Metallothionein induction is not involved in cadmium accumulation in the duodenum of mice and rats fed diets containing high-cadmium rice or sunflower kernels and a marginal supply of zinc, iron, and calcium. Journal of Nutrition. 135:99-108. **0000168358**
7. Reeves, P.G., DeMars, L.C.S., Johnson, W.T., Lukaski, H.C. 2005. Dietary copper deficiency reduces iron absorption and duodenal enterocyte hephaestin protein in male and female rats. Journal of Nutrition. 135:92-98. **0000169586**
8. Reeves, P.G., DeMars, L.C.S. 2005. Repletion of copper-deficient rats with dietary copper restores duodenal hephaestin protein and iron absorption. Experimental Biology and Medicine. 230:320-325. **0000174739**
9. Chaney, R.L., Reeves, P.G., Ryan, J.A., Simmons, R.W., Welch, R.M., Angle, J.S. 2004. An improved understanding of soil cd risk to humans and low cost methods to remediate soil cd risks. Biometals. 17(5):549-553. **0000161735**
10. Finley, J.W. 2005. Selenium accumulation in plant foods. Nutrition Reviews. 63(6):196-202. **0000172418**
11. Hintze, K.J., Wald, K., Finley, J.W. 2005. Phytochemicals in broccoli transcriptionally induce thioredoxin reductase. Journal of Agricultural and Food Chemistry. 53:5535-40. **0000174701**
12. Hunt, J.R. 2004. Combating iron deficiency - supplementation, fortification, and dietary tactics [abstract]. The Journal of Trace Elements in Experimental Medicine. 17(4):259. **0000166340**
13. Beiseigel, J.M., Hunt, J.R. 2005. Algorithms for estimating zinc absorption from whole diets [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(4):A456. **0000171309**
14. Hunt, J.R., Swain, J.H. 2005. Bioavailability to humans of an electrolytic elemental iron fortificant, assessed after radiolabeling by neutron activation [abstract]. The Federation of American Societies for Experimental Biology **0000171398**

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Journal. 19(5):A1468.

15. Hadley, K.B., Johnson, L.K., Hunt, J.R. 2005. Serum prohepcidin does not predict iron absorption in healthy women [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1481. 0000171285

16. Hunt, J.R., Hadley, K.B., Johnson, L.K. 2005. Serum prohepcidin was not associated with iron absorption by healthy women in a dose-response assessment of elemental iron powders [abstract]. First Congress of the International BioIron Soiety. p. 57. 0000176519

17. Reeves, P.G., DeMars, L.C. 2005. A chronological study on the effects of Cu deficiency on Fe absorption and metabolism in male rats [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1485. 0000171174

18. Reeves, P.G., DeMars, L.C. 2005. Low Fe absorption and signs of anemia in Cu-deficient rats are reversed by short-term dietary supplementation with Cu [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1485. 0000171178

19. Chaney, R.L., Reeves, P.G., Ryan, J.A. 2004. Risk assessment for cadmium in phosphate fertilizers [abstract]. American Chemical Society Abstracts. (228th ACS National Meeting, Philadelphia, Pennsylvania, August 22-26, 2004). 0000167877

20. Park, S.Y., Woodward, C.L., Birkhold, S.G., Kubena, L.F., Nisbet, D.J., Ricke, S.C. 2004. The combination of zinc compounds and acidic pH limits aerobic growth of a Salmonella typhimurium poultry marker strain in rich and minimal media. Journal of Environmental Science and Health. B39:199-207. 0000171672

21. Reeves, P.G. 2005. Iron absorption and intestinal hephaestin protein in copper-deficient rats [abstract]. Presented at Gordon Research Conference on Cell Biology of Metals. Bates College, Lewiston, ME. July 3-8, 2005. 0000177803

Approved: CHANDLER LAURENCE D **Date:** 09/20/2005

Project Number: 5450-51000-035-06S **Accession:** 0404307 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH

Start Date: 03/01/2001 **Term Date:** 09/15/2005

National Programs: 107 N Human Nutrition

Title: AGRICULTURAL PRODUCTION ASPECTS OF HIGH SELENIUM MEAT AND WHEAT

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-1-0310

Organization Name: **NORTH DAKOTA STATE UNIVERSITY**

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

Selenium-enriched beef may be an ideal means of supplementing dietary selenium. A series of studies have demonstrated that it is possible to enrich beef with selenium by incorporating grains and hay grown on selenium-rich soils into feedlot rations of cattle. Studies have demonstrated that selenium in beef can be increased from an average of 0.2 parts per million (ppm) to as much as 2.5 ppm. Further, feedlot feeding of these rations did not result in any signs or symptoms of selenium toxicity. Recent studies have concentrated on how precisely the levels of selenium in beef can be modulated by feeding selenium-enriched feedstuffs.

4b. List other significant accomplishments, if any.

None

4c. List any significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under a reimbursable agreement number 58-5450-1-0310 between ARS and the Department of Animal Science, North Dakota State University. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

The overall project is doing a comprehensive study of how high-Se wheat, meat and broccoli are produced and utilized by animals. The Animal Science portion of the project is being conducted at North Dakota State Unversity.

Scientific Publications: **Log 115:**

1. Taylor, J.B., Finley, J.W., Caton, J.S. 2005. Effect of the chemical form of supranutritional selenium on selenium load and selenoprotein activities in virgin, pregnant, and lactating rats. Journal of Animal Science. 83:422-429. **0000159747**

Approved: CHANDLER LAURENCE D **Date:** 10/21/2005

Project Number: 5450-51000-035-08S **Accession:** 0404351 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: MARY J KRETSCH
Start Date: 01/01/2001 **Term Date:** 06/30/2005

National Programs: 107 N Human Nutrition

Title: HEALTH BENEFITS OF HIGH-SELENIUM FOODS TO HUMANS

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-1-0315

Organization Name: **OREGON STATE UNIVERSITY**

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

The distribution of selenium between proteins found in blood is an indication of how Se is metabolically partitioned in the body. Selenium is primarily found in three proteins: serum albumin, and the selenoproteins plasma glutathione peroxidase and plasma selenoprotein P. Ongoing collaborative studies with Oregon State University have been using a chromatographic method developed at OSU to separate these proteins. Samples from human trials, as well as from animal trials, have been analyzed by this procedure. The analyses are almost complete and the results are being incorporated into pertinent manuscripts.

4b. List other significant accomplishments, if any.

None

4c. List any significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under a reimbursable agreement number 58-5450-1-0315 between ARS and the Department of Environmental and Molecular Toxicology, Oregon State University. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

Research is being conducted on Se metabolism, and especially how different forms of Se affect the use and distribution of Se in the body.

Scientific Publications: **Log 115:**

Approved: CHANDLER LAURENCE D **Date:** 10/21/2005

Project Number: 5450-51000-035-09S **Accession:** 0404746 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH

Start Date: 06/01/2001 **Term Date:** 05/31/2006

National Programs: 107 N Human Nutrition

Title: HEALTH BENEFITS OF INTERACTING PHYTOCHEMICALS IN BROCCOLI

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-1-0330

Organization Name: UNIV OF ILLINOIS

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

Selenium-enriched broccoli may be an excellent supplemental source of selenium because it contains chemical forms of selenium that protect against cancer but do not accumulate in the body as well as other forms of broccoli. A clinical study fed volunteers either 9 or 90 grams of broccoli either enriched or unenriched in selenium each day for 2 months. When compared to a similar study that fed subjects high-selenium wheat cereal, selenium from broccoli accumulated approximately 50% less than selenium from wheat, and more selenium from broccoli was excreted in the urine. These results support the hypothesis that selenium-enriched broccoli may supply cancer-inhibiting metabolites without inducing excessive accumulation in the body

4b. List other significant accomplishments, if any.

None

4c. List any significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under a reimbursable agreement number 58-5450-1-0330 between ARS and the Department of Chemistry, University of North Dakota. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

The collaborator at the Univ. Of Illinois has an active research program examining the health benefits of glucosinolate compounds found in broccoli. She has collaborated with a plant breeder to develop different strains of broccoli with different concentrations of glucosinolates. Our work with broccoli has been examining the health benefits of selenium that accumulates in broccoli grown under special high-Se conditions. This project is designed to join these two lines of research and determine whether the two compounds have any nutritional interaction.

Scientific Publications: **Log 115:**

Approved: CHANDLER LAURENCE D **Date:** 09/23/2005

Project Number: 5450-51000-035-10G **Accession:** 0404556 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: MARY J KRETSCH
Start Date: 06/07/2001 **Term Date:** 06/06/2005

National Programs: 107 N Human Nutrition

Title: USE OF GEOLOGICAL INFORMATION SYSTEM TO VISUALIZE HIGH SELENIUM SOIL AND CROPS

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 59-5450-1-0325

Organization Name: **UNIV OF NORTH DAKOTA**

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

Understanding the geological, geographical and soil-related factors that result in the development of high-Se soils is essential to understanding why certain crops accumulate selenium. A comprehensive study of the factors affecting selenium accumulation in soils and pasture grasses was undertaken in a seleniferous area of South Dakota. A series of soil samples were taken across traverse lines that went from low to very high selenium content; soil moisture samplers were placed in the same areas; samples were taken across two consecutive growing seasons. Multiple chemical analyses were performed and statistical models were used to develop correlates with selenium concentrations. Total selenium was correlated to total organic carbon and water soluble selenium was best correlated to sodium, magnesium and sulfate. In general, the variation in soil selenium concentrations was primarily in the soluble selenium component.

4b. List other significant accomplishments, if any.

None

4c. List any significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under reimbursable agreement number 59-5450-1-0325 between ARS and the Department of Geology, University of North Dakota. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

The purpose of this project is to examine the geological, geographical and soil-related factors that result in the development of high-Se soils and the subsequent production of high-Se crops. Knowledge of these factors is essential as producers ever attempt to market high-Se wheat on a regular basis.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 10/13/2005

Project Number: 5450-51000-035-11S **Accession:** 0404830 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: MARY J KRETSCH
Start Date: 09/01/2001 **Term Date:** 08/31/2006
National Programs: 107 N Human Nutrition
Title: SELENIUM SUPPLEMENTATION OF SUBJECTS WITH EXTREMELY LOW SELENIUM INTAKES
Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** Yes
Terminate in Two Months? No
Agreement Number: 58-5450-1-0163F
Organization Name: INST OF NUTRITION & FOOD HYGIENE

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

Bioavailability of selenium to humans is best assessed by feeding a source of selenium to selenium-deficient humans and measuring the change in selenium responsive biomarkers (e.g. selenoprotein activity and selenium concentrations). High-selenium beef, wheat and broccoli were used to make a ready to eat, dehydrated soup mix. The soup mix was then fed in a randomized design to selenium-deficient subjects living in the mountains of south-central China. Bioavailability was assessed by measuring changes in glutathione peroxidase, tissue selenium concentrations and retention of a stable isotope of selenium. Preliminary results demonstrate that all foods raise peroxidase activity and tissue selenium concentrations, but selenium-enriched beef may be the single most effective food form.

4b. List other significant accomplishments, if any.

Selenium accumulates in the brain and limited data suggests that supplemental selenium improves neuropsychological function. As part of the feeding study conducted in DaChang, China, subjects were administered a battery of cognitive, neurological and psychological tests. Tests are now being analyzed and results will be used to determine whether selenium supplementation of deficient individuals is necessary for optimal brain function.

4c. List any significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under reimbursable agreement number 58-5450-1-0163F between ARS and the Chinese Academy of Preventive Medicine. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

An overall objective in our laboratory is to determine whether foods enriched in Se can safely and effectively be utilized as supplemental sources of Se. To this end we are conducting field studies that are examining the geological and biological processes that cause accumulation of Se in soil, plants and animals. We also are conducting basic nutritional trials in animals that are determining whether Se from wheat, meat and broccoli is effective for a number of health-related problems such as prevention of colon cancer. The ultimate measure of the "usefulness" of these

Project Number: 5450-51000-035-11S **Accession:** 0404830 **FY:** 2005

food sources of Se is to feed them to a target population and measure basic aspects of health and metabolism.

This project is feeding high-Se broccoli, wheat and meat to healthy humans living in a low-Se area of China. We have chosen to study this area of China because Se intakes there are the lowest in the world.

Scientific Publications: **Log 115:**

Approved: CHANDLER LAURENCE D **Date:** 10/03/2005

Project Number: 5450-51000-035-13T **Accession:** 0405631 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH

Start Date: 07/01/2002 **Term Date:** 07/31/2006

National Programs: 107 N Human Nutrition

Title: COMBATING IRON DEFICIENCY: ABSORPTION & EFFICACY IN HUMANS OF ELEMENTAL IRON POWDERS & HEME IRON

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 03-5450-3-0237

Organization Name: COOPERATIVE STATE RESEARCH EDUCATION & EXTENSION SERVICE (CSREES), U.S. DEPARTMENT OF AGRICULTURE

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

None

4b. List other significant accomplishments, if any.

None

4c. List any significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under trust agreement 03-5450-3-0237, concerning the USDA CSREES Nutritional Research Initiative competitive grant 2002-01885 (and tracked by CREES as CRIS 0192629) entitled "Combating Iron Deficiency: Absorption and Efficacy in Humans of Elemental Iron Powders and Heme Iron". Additional information can be found in the parent CRIS project 5450-51000-035-00D.

This project has two objectives: 1) to determine the efficacy of fortifying food with elemental iron powders, relative to equivalent amounts of ferrous sulfate, or of supplementing with a limited amount of iron in the heme form, on serum ferritin in women with low iron stores, and 2) to determine the absorption of irradiated electrolytic iron powder, relative to ferrous sulfate, as affected by dose and by interactions with ascorbic acid and phytic acid.

This year we report the results of the second objective. In 3 experiments, iron absorption was measured in 56 volunteers, using Fe-59 labeled ferrous sulfate, and an electrolytic iron powder that had been labeled with Fe-55 by neutron activation several years earlier. The iron absorbed from the irradiated electrolytic powder was much lower than expected, 5-10% that of ferrous sulfate, in contrast with earlier results (J Nutr 1999;129:181-7) using this same iron powder, which showed retention of 50% relative to ferrous sulfate in pigs. This observed low absorption was also in contrast to the efficacy results (with non-irradiated powder) in objective 1, as reported last year. This raises the possibility that the heat of the irradiation process had reduced the bioavailability, and that pigs may not be a good model of human iron absorption. Ascorbic acid enhanced iron absorption from ferrous sulfate

Project Number: 5450-51000-035-13T **Accession:** 0405631 **FY:** 2005

more than from electrolytic powder, and a higher administered iron dose reduced iron absorption from ferrous sulfate more than from electrolytic. Phytic acid inhibition did not significantly interact with the iron source, but this was likely because the inhibition was extensive, with a meal that was already poorly bioavailable. The results support the conclusion that the reduced bioavailability of some elemental iron powders (previously shown to correlate with the solubility of the powder) renders them less interactive with other enhancers and inhibitors of iron absorption.

Additional data analysis and manuscript preparation is in progress.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/23/2005

Project Number: 5450-51000-035-16S **Accession:** 0405998 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: MARY J KRETSCH
Start Date: 09/16/2002 **Term Date:** 09/15/2007

National Programs: 107 N Human Nutrition

Title: WHOLE BODY COUNTING AND RADIOTRACER METHODS TO SUPPORT RESEARCH ON HUMAN MINERAL NUTRITION

Period Covered From: 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-2-0335

Organization Name: UNIV OF NORTH DAKOTA

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?

3b. List the milestones that you expect to address over the next 3 years (FY 2006, 2007, and 2008). What do you expect to accomplish, year by year, over the next 3 years under each milestone?

4a. What was the single most significant accomplishment this past year?

None

4b. List other significant accomplishments, if any.

None

4c. List any significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under Specific Cooperative Agreement #58-5450-8-111 between ARS and University of North Dakota Physics Department. Additional details of the research can be found in the reports for the parent CRIS project 5450-51000-035-00D, Mineral Utilization and Bioavailability in the 21st Century, with Changing Diets and Agricultural Practices and related CRIS project 5450-51000-034-00D, Mineral Intakes for Optimal Bone and Joint Development and Health.

To make dietary recommendations and evaluate dietary practices that promote good mineral nutrition for the population, there must be sensitive methods for measuring mineral nutrient absorption, excretion, retention, and food bioavailability. The use of isotopic tracer methodology can effectively contribute to meeting these needs. Specifically, use of a whole body scintillation counter can safely and sensitively determine whole body retention of mineral elements that have gamma-emitting isotopes with short to moderate half-lives, such as cadmium, calcium, copper, iron, magnesium, manganese, and zinc. The whole body counting approach has

Project Number: 5450-51000-035-16S **Accession:** 0405998 **FY:** 2005

the advantage of determining mineral retention without volunteer inconvenience, high variability, and incomplete sample collections associated with collecting mineral excretion data. It allows the use of a true "tracer" that does not alter the absolute mass of the mineral under investigation, and is easily and sensitively measured with minimal labor. This agreement provides the expertise of a certified health physicist to cooperate with nutrition scientists at the Grand Forks Human Nutrition Research Center, applying an interdisciplinary approach to answering nutrition questions with whole body counting methodology.

Accomplishments this year included health physicist support of investigations to assess bioavailability of calcium, iron, and zinc using gamma-emitting radiotracers in human absorption studies. The health physicist led the work of the FDA-sanctioned UND Radioactive Drug Research Committee, which reviewed and provided oversight for all nutrition experiments employing radioactive tracers.

This year, radioactive tracers were used to assess women's absorptive adaptation to different dietary zinc intakes from low to recommended levels, as a possible indicator of zinc requirements and to further assess women's absorptive adaptation to supplemental zinc. Review and radiological approvals were obtained for studies to assess the bioavailability of iron from different staple food varieties and to assess the interaction of calcium and phytic acid on zinc absorption.

In addition, an evaluation of over 15 years of UND Radon Monitoring Facility data was extended. It was previously found that radon concentrations in Grand Forks homes paralleled the body activity of bismuth-214 (a decay product of radon) in community volunteers, with the greatest activity in the fall quarter and least in the spring quarter. These seasonal variations are consistent with increased body radon accumulation in enclosed buildings, and possibly increased body fat (sites of physiological radon storage) during winter months. Further analysis of this data was consistent with other reports of accumulation of radon in fatty tissue: a comparison of the mean 214Bi activity measured in humans with the mean bedroom radon concentration was consistent with a 7-fold accumulation of radon in the body fat of subjects. This accumulation of radon in fatty tissue may partially account for an increased cancer risk associated with obesity.

The sensitive detection of gamma-emitting isotopic tracers provides uniquely sensitive measurements of mineral retention in humans and animal models, as affected by nutritional status, dietary sources of nutrients, and genotype.

Scientific Publications: **Log 115:**

Approved: CHANDLER LAURENCE D **Date:** 09/23/2005

Project Number: 5450-51000-035-17T **Accession:** 0406927 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH

Start Date: 01/01/2003 **Term Date:** 12/31/2007

National Programs: 107 N Human Nutrition

Title: NUTRITIONAL VALUE OF BUCKWHEAT: TRACE ELEMENT VARIABILITY AND BIOAVAILIBILITY AND FAGOPYRITOL CONTENT

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-3-0406

Organization Name: MINN-DAK GROWERS, LTD.

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

North Dakota produces the majority of buckwheat grown in the US and much of that is grown in areas known to have high-selenium soils. A survey of buckwheat grown in these areas has shown it to contain between 0.5 and 1.0 micrograms selenium per gram, making it a nutritionally important source of selenium. However the bioavailability of selenium from buckwheat is not known. To determine the bioavailability of selenium from buckwheat we repleted the selenium status of selenium-deficient rats with buckwheat enriched in selenium. Other rats were repleted with the pure the chemical forms of selenium, selenomethionine and selenite. After 49 days of selenium repletion, rats were killed and selenium status was determined by measuring selenoprotein activities and selenium concentrations in important tissues. Initial analyses (analyses are ongoing) showed that selenium from buckwheat was only 45-60% as bioavailable as selenomethionine and selenite. However, the average concentration of selenium in North Dakota buckwheat is almost twice the average concentration in US cereal grains, thus selenium-enriched buckwheat is an important dietary source of selenium, despite slightly lower bioavailability.

4b. List other significant accomplishments, if any.

The bioavailability of selenium in protein-rich buckwheat flour was studied by using selenium deficient rats. The AIN-93 diet was optimized for induction of selenium deficiency, and rats were fed this diet for sufficient time to induce deficiency. Selenium bioavailability from buckwheat was studied by cooking and lyophilizing buckwheat flour, incorporating this into diets and then comparing the response of selenium-sensitive indicators to animals fed purified forms of selenium. Using this procedure, selenium in buckwheat flour was shown to have relatively low bioavailability.

4c. List any significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under Specific Cooperative Agreement number 58-5450-3-0406 between ARS and MinDak Growers, Ltd. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

Project Number: 5450-51000-035-17T **Accession:** 0406927 **FY:** 2005

The purpose of this agreement is to study the nutritional value of buckwheat, with emphasis on buckwheat as a source of nutritionally essential minerals. MinDak Growers, Ltd. is collecting buckwheat and soil samples from buckwheat producers, and preparing those samples for analysis. The GFHNRC is conducting all chemical analyses, and is planning and conducting studies in rats to determine the bioavailability of minerals from buckwheat.

Scientific Publications: **Log 115:**

1. Leary, P.D., Badaruddin, M., Finley, J.W., Reeves, P.G. 2005. Bioavailability of selenium from buckwheat bran [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(4):A93. **0000171617**

Approved: ROOS ERIC E **Date:** 10/03/2005

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH

Start Date: 09/29/2003 **Term Date:** 09/28/2008

National Programs: 107 N Human Nutrition

Title: HUMAN STUDIES RESEARCH

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-3-0324

Organization Name: **UNIV OF NORTH DAKOTA**

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?**

   To improve the nation's nutrition and health (Goal 4 of the Agricultural Research Service Strategic Plan, 2003-2007), research is necessary to generate new knowledge in human nutrition, to improve the understanding of optimal nutrient requirements for known and new classes of nutrients at all stages of the life cycle, and to better understand relationships between diet and health. Although much can be learned using basic biochemical, cellular, and animal models of human nutrition, some questions require direct human studies to assure that the results are applicable to humans.

This agreement between ARS and UND is based on a mutual interest in human nutrition as it pertains to the maintenance of optimum health, including reduction of disease risk. The objective of the agreement is to investigate the role of nutrients in human health, to determine their bioavailability from foods and mixed diets, to investigate their biological activities in cancer prevention, in bone and joint health, in cardiovascular health, and in physiological and psychological development and function. Diets designed to contain known amounts of essential and non-essential nutrients, or foods containing specific nutrients or other bioactive components, are provided to human volunteers under controlled conditions of dietary intake, so that their effects on clinical chemical (blood constituents), physiological (blood pressure, cardiovascular function respiratory function, neuro-muscular function), neurological (mood, neurologic function), urinary and fecal excretion, and other measures of biological activity and health status may be determined. Human studies include residential, non-residential and field-based investigations using such approaches as dietary recall, metabolic balance, radio/stable isotope retention, physiological/neurological function assessment, and specific metabolic/enzyme analyses. UND provides an Institutional Review Board to review all protocols for studies involving human subjects at the Grand Forks Human Nutrition Research Center, provides the competent personnel to participate in the planning and execution of studies, collaborates with ARS on related animal model studies, and provides peer review of scientific manuscripts.

This research is directly related to five components of the ARS National Human Nutrition 107 research plan: (1) Human Nutrition Requirements, (2) Diet, Genetics, Lifestyle, and the Prevention of Obesity and Disease, (5) Health Promoting Intervention Strategies for Targeted Populations (6) Health Promoting Properties of

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2005

Plant and Animal Foods, and (7) Bioavailability of Nutrients and Food Components. The research provides knowledge to policy makers and other scientists for setting guidelines to improve human nutrition and reduce disease risk.

2. **List the milestones (indicators of progress) from your Project Plan.**

This specific cooperative agreement provides support for human studies. Specific milestones are documented in the CRIS projects cited above. In support of those milestones, this report addresses milestones for conducting key GFHNRC human studies with approval by the UND IRB.

Year 1 (FY2004)

Collaborate in planning and approvals for human studies
Recruit subjects and begin or continue data collection for approved studies
006 Human absorption of iron fortification sources assessed using an isotope displacement method
007 Beef as a component of a healthy diet: does it improve selenium and trace element status of healthy men and women?
049 Body iron excretion
144 Effects of low, moderate and high zinc intakes on copper nutriture
403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women?
Complete data collection for approved studies
064 Meat protein and calcium: Do they interact synergistically or antagonistically?
098 Effect of daily intake of wheat cereal containing various concentrations of selenium on selenium status in humans
501 Improved health in humans consuming broccoli high in sulforaphane and selenium
404 Physical health, fitness, nutrition, and mental health in Northern Plains Indians

Year 2 (FY2005)

Collaborate in planning and approvals for human studies
008 Beans and colon health in humans: Effect of resistant starch from beans and changing the lower bowel bacterial populations and the production of short chain fatty acids
042 Effects of calcium and phytate on zinc absorption
095 Iron absorption from agricultural products
298 Micro foundations of health and economic development: The impact of iron supplementation on social and economic prosperity in Indonesia
Recruit subjects and begin or continue data collection for approved studies
006 Human absorption of iron fortification sources assessed using an isotope displacement method
008 Beans and colon health in humans: Effect of resistant starch from beans and changing the lower bowel bacterial populations and the production of short chain fatty acids
049 Body iron excretion
095 Iron absorption from agricultural products
403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women?
Complete data collection for approved studies
007 Beef as a component of a healthy diet: does it improve selenium and trace element status of healthy men and women?

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2005

090 Determining dietary zinc requirements from adaptation in zinc absorption
144 Effects of low, moderate and high zinc intakes on copper nutriture

Year 3 (FY2006)

Collaborate in planning and approvals for human studies
009 Predicting dietary selenium needs to achieve target blood selenium levels
303 Estimating mineral nutrient requirements by cross-sectional statistical analyses of existing data
Recruit subjects and begin or continue data collection for approved studies
009 Predicting dietary selenium needs to achieve target blood selenium levels
042 Effects of calcium and phytate on zinc absorption
095 Iron absorption from agricultural products
403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women?
601 Nutrition effects on cognitive performance of young versus elderly community-living adults
Complete data collection for approved studies
008 Beans and colon health in humans: Effect of resistant starch from beans and changing the lower bowel bacterial populations and the production of short chain fatty acids
042 Effects of calcium and phytate on zinc absorption
095 Iron absorption from agricultural products
298 Micro foundations of health and economic development: The impact of iron supplementation on social and economic prosperity in Indonesia

Year 4 (FY2007)
Collaborate in planning and approvals for human studies
Recruit subjects and begin or continue data collection for approved studies
009 Predicting dietary selenium needs to achieve target blood selenium levels
Complete data collection for approved studies
403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women?

3a. List the milestones that were scheduled to be addressed in FY 2005. For each milestone, indicate the status: fully met, substantially met, or not met. If not met, why.

1. Collaborate in planning and approvals for human studies.

   008 Beans and colon health in humans: Effect of resistant starch from beans and changing the lower bowel bacterial populations and the production of short chain fatty acids

   Milestone Fully Met

2. 042 Effects of calcium and phytate on zinc absorption

   Milestone Fully Met

3. 095 Iron absorption from agricultural products

   Milestone Fully Met

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2005

3a. 4. 298 Micro foundations of health and economic development: The impact of iron supplementation on social and economic prosperity in Indonesia

Milestone Fully Met

5. Recruit subjects and begin or continue data collection for approved studies

006 Human absorption of iron fortification sources assessed using an isotope displacement method

Milestone Fully Met

6. 008 Beans and colon health in humans: Effect of resistant starch from beans and changing the lower bowel bacterial populations and the production of short chain fatty acids

Milestone Fully Met

7. 049 Body iron excretion

Milestone Fully Met

8. 095 Iron absorption from agricultural products

Milestone Fully Met

9. 403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women?

Milestone Fully Met

10. Complete data collection for approved studies

007 Beef as a component of a healthy diet: does it improve selenium and trace element status of healthy men and women?

Milestone Fully Met

11. 090 Determining dietary zinc requirements from adaptation in zinc absorption

Milestone Fully Met

12. 144 Effects of low, moderate and high zinc intakes on copper nutriture

Milestone Fully Met

3b. List the milestones that you expect to address over the next 3 years (FY 2006, 2007, and 2008). What do you expect to accomplish, year by year, over the next 3 years under each milestone?

FY2006
Collaborate in planning and approvals for human studies (Specific objectives are added in support of the CRIS research projects or associated, externally-funded projects as planned and approved through the UND Institutional Review Board)
009 Predicting dietary selenium needs to achieve target blood selenium levels. An assessment of the relationship between selenium supplement dose and blood selenium response will be useful for developing recommendations for selenium supplementation to reduced cancer risk.
303 Estimating mineral nutrient requirements by cross-sectional statistical analyses of existing data. Data combined from past mineral balance studies will have

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2005

substantial statistical power to evaluate trace mineral requirements and indices of marginal status in healthy adults.
Recruit subjects and begin or continue data collection for approved studies
009 Predicting dietary selenium needs to achieve target blood selenium levels (see above)
042 Effects of calcium and phytate on zinc absorption. This study will determine the interaction of dietary calcium and phytate on zinc absorption in practical human diets.
095 Iron absorption from agricultural products in an assessment of iron absorption from different strains of Nigerian corn and from bean varieties with different colors and polyphenol profiles, differences from in vitro Caco-2 cell bioavailability measurements can be validated with human absorption data.
403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women? This 2-y supplementation study will determine whether zinc and copper, in addition to calcium supplementation, will increase bone mineral density.
601 Nutrition effects on cognitive performance of young versus elderly community-living adults. This study will evaluate age-related changes in memory performance, as modified by factors including dietary intake and blood nutrient concentrations.
Complete data collection for approved studies
008 Beans and colon health in humans: Effect of resistant starch from beans and changing the lower bowel bacterial populations and the production of short chain fatty acids. This evaluation of the effect of bean consumption on fecal substrates and their metabolism by intestinal flora may provide data on the benefits of beans related to cancer risk factors.
042 Effects of calcium and phytate on zinc absorption (see above)
095 Iron absorption from agricultural products (see above)
298 Micro foundations of health and economic development: The impact of iron supplementation on social and economic prosperity in Indonesia Participation in this UCLA-based study to provide biochemical assessment of iron status, will help assess the impact of iron supplementation on family economic status in Indonesia.

(FY2007)
Collaborate in planning and approvals for human studies
Recruit subjects and begin or continue data collection for approved studies
009 Predicting dietary selenium needs to achieve target blood selenium levels (see above)
Complete data collection for approved studies
403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women? (see above)

4a. What was the single most significant accomplishment this past year?

Iron absorption measurements with 28 healthy premenopausal women, were used to assess the relationship of iron absorption to hepcidin, a recently discovered peptide with antimicrobial properties, which is proposed to play a central role in the biological regulation of iron absorption. In contrast with serum ferritin, serum pro-hepcidin concentrations were unrelated to iron absorption in these healthy women. Such studies can contribute to a better understanding of the control of human iron absorption. See additional information in the report for CRIS 5450-51000-035-00D.

4b. List other significant accomplishments, if any.

Major accomplishments are also described in the reports for the CRIS projects listed above, and include:

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2005

- Iron absorption studies (3 experiments involving 56 volunteers) that showed that the reduced bioavailability of some elemental iron powders (previously shown to correlate with the solubility of the powders) renders them less interactive with other enhancers and inhibitors of iron absorption, such as ascorbic acid and phytic acid. See additional information in the report for CRIS 5450-51000-035-00D and 5450-51000-035-13T.
- A zinc supplementation study involving 209 students in the 7th grade found that the addition of 20 mg zinc to juices administered on school days for 10 weeks improved visual memory reaction times, word recognition, and vigilance in cognitive testing. For more information, see the report for CRIS 5450-51530-009-00D.
- A controlled diet study in which 9 men consumed adequate and then marginal copper (1.4 vs. 0.7 mg/2500 kcal/d) for 7 weeks each indicated that marginal dietary copper decreases muscle cytochrome c oxidase activity and impairs cardiorespiratory function during submaximal exercise. For more information, see the report for CRIS 5450-51530-009-00D.

4c. List any significant activities that support special target populations.

A partnership between American Indians, tribal nations, and the USDA, ARS, initially developed in FY2004, enabling research on preventive measures to reduce health problems such as obesity and diabetes among American Indians in the Northern Plains was further developed by collecting and analyzing the nutrient content of traditional native foods, and by providing learning experiences in nutrition research for student interns from reservations in North Dakota and Arizona (see 5450-51530-009-00D).

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Discovered that a high, compared with a low meat diet improved calcium retention in postmenopausal women when calcium intake was low, but had no minimal when calcium intake was high.
Determined that soy protein (substituted for meat protein) had a negligible effect on calcium retention by postmenopausal women.
Determined that iron absorption of fortified foods did not differ significantly between genetic carriers (~10% of US population) of a mutation associated with hemochromatosis and those without the mutation.
Showed that ferrous sulfate, reduced, electrolytic, and heme iron can all improve body iron status in premenopausal women.
Conducted a survey assessing Native American health, nutrition and physical activity and distributed summaries of the findings to participating Indian tribes and communities.

6. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?

The accomplishments are documented in manuscripts in peer reviewed scientific journals, and in presentations at scientific and public meetings. See the reports for the CRIS projects listed above.

7. List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below).

See the reports for the CRIS projects listed above.

Scientific Publications: **Log 115:**

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2005

Approved: CHANDLER LAURENCE D **Date:** 10/27/2005

Project Number: 5450-51000-035-19S **Accession:** 0408261 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: MARY J KRETSCH
Start Date: 04/16/2004 **Term Date:** 03/15/2008

National Programs: 107 N Human Nutrition

Title: DEVELOPMENT OF RAPID SELENIUM ANALYSIS TECHNOLOGY

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-4-0346

Organization Name: UNIV OF NORTH DAKOTA

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

Work has been performed to develop a prototype for rapidly analyzing selenium in wheat, buck wheat, and mustard. This involved development of a sample treatment method, fabrication of a prototype instrument, and laboratory and field (mill) testing of prototype. The sample treatment step proved to be the most critical one for this application. Multiple strategies were pursued, resulting in a feasible method being developed to extract selenium from the biomass and convert it to the (+IV) oxidation state for analysis by hydride generation atomic absorption spectroscopy. Initial studies were conducted to determine whether hot, pressurized water could be used to extract Se from wheat. Although conditions were identified that efficiently liquefied wheat samples, the extracts carried a significant organic load. The breakthrough came when a mixture of nitric acid and hydrogen peroxide mixture was used: it entirely decomposes the organic matrix under the same hot, pressurized conditions, yielding in less than 10 min. a colorless solution without appreciable loss of selenium. Compared to microwave-assisted and UV light-assisted digestion, this method appears to have the best possibility of commercial viability.
Nitrites evolved during the digestion by this method interfere with the analysis of selenium. To address this problem, the digested sample is treated with sulfamic acid, which converts nitrate to sulfate, nitrogen and water. The complete method involves two reaction steps of approximately 10 min. each and which yields a liquid containing all of the selenium from the original biomass in the (+IV) state, i.e., in a form suitable for subsequent analysis.
This technology was incorporated in a prototype instrument consisting of six subsystems: chemical reagent storage and supply system; 1st stage reaction chamber; 2nd stage reaction chamber; hydride generation system; atomic absorption spectrophotometer; a computer automation system. The prototype was installed for testing at the Minn-Dak Growers mill, Grand Forks, ND. The major problems encountered were: thermal failures in interconnecting polymer tubing (addressed by replacement with stainless steel, although the corrosion rate of that material is too high for long-term use); insufficient capacity of original computer system for the service load; reaction chamber screw fitting subject to cross-thread damage; inconsistent accuracy and precision. Efforts are underway to address these problems in the improved prototype device.

4b. List other significant accomplishments, if any.

None.

Project Number: 5450-51000-035-19S **Accession:** 0408261 **FY:** 2005

4c. List any significant activities that support special target populations.

None.

4d. Progress report.

This report serves to document research conducted under 58-5450-4-0346, specific cooperative agreement between ARS and the University of North Dakota Chemistry Department. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

The purpose of this agreement is to develop a practical means of rapidly determining the selenium contents of small grains for ultimate use in sorting grains on the basis of selenium content for the development of high-selenium grain products. Scientists at the University of North Dakota are developing the sample liquification methology and the computer control of the prototype instrument and are field testing that prototype. The GFHNRC is conducting confirming chemical analyses of reference materials for the testing of the methods and the evaluation of the prototype.

Scientific Publications: **Log 115:**

Approved: CHANDLER LAURENCE D **Date:** 09/23/2005

Project Number: 5450-51000-035-21N **Accession:** 0408588 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 03/15/2004 **Term Date:** 03/14/2009
National Programs: 107 N Human Nutrition
Title: SELENIUM ACCUMULATION IN VEGETABLES
Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No
Agreement Number: 58-5450-4-0163FN
Organization Name: **NEW ZEALAND INS. CROP & FOOD RES**

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

Residents of New Zealand have some of the lowest selenium intakes among all Western nations. Enhancing the selenium content of vegetables may be an ideal means of improving the dietary selenium supply. Broccoli, squash and tomato varieties were tested for the ability to take up selenium, and the chemical form of selenium was examined in these plants. Certain varieties of broccoli and squash accumulated large amounts of selenium; the chemical form of selenium varied between varieties. These preliminary data have been used to select the most promising varieties for further and more extensive testing.

4b. List other significant accomplishments, if any.

None

4c. List any significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under a Memorandum of Understanding number 58-5450-4-0163FN between ARS and the New Zealand Institute of Crop and Food Research. Additional details of this research can be found in the report for the parent CRIS 5450-51000-035-00D.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/23/2005

Project Number: 5450-51000-035-22T **Accession:** 0408646 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH

Start Date: 05/01/2004 **Term Date:** 04/30/2009

National Programs: 107 N Human Nutrition

Title: HIGH SELENIUM PINTO BEANS AS A VALUE-ADDED PRODUCT

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 04-5450-4-0418

Organization Name: **NORTHARVEST BEAN GROWERS ASSOCIATION**

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

Dry edible beans are a major crop raised in North Dakota, and there may be substantial health benefits associated with consumption of beans. Moreover, beans raised in North Dakota may contain much higher concentrations of selenium, a nutrient that may help prevent cancer. Representative samples of harvested pinto beans obtained from more than 70 locations in five distinct geographical regions of North Dakota were analyzed for selenium and other major mineral elements. The average content of selenium was approximately 5-fold the U.S. average, but there was great variation, even within a small geographical area. Modeling procedures are being used to determine variables that may be predictive of selenium content; the project will be extended over a second growing season.

4b. List other significant accomplishments, if any.

None

4c. List any significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under a Trust Agreement number 04-5450-4-0418 between ARS and NorthHarvest Bean Growers, Ltd. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

The purpose of this agreement is to determine factors that affect the accumulation of selenium in pinto beans.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/23/2005

Project Number: 5450-51000-035-23T **Accession:** 0408799 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH

Start Date: 07/15/2004 **Term Date:** 03/31/2006

National Programs: 107 N Human Nutrition

Title: COMPARISON OF IRON BIOAVAILABILITY TO HUMANS FROM TWO STRAINS OF CORN USED IN NIGERIA

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 05-5450-5-0171

Organization Name: INTERNATIONAL INSTITUTE OF TROPICAL AGRICULTURE

Progress and Outcomes:

4a. **What was the single most significant accomplishment this past year?**

None

4b. **List other significant accomplishments, if any.**

None

4c. **List any significant activities that support special target populations.**

None

4d. **Progress report.**

This report serves to document research conducted under trust agreement 58-5450-4-171F between ARS and the International Institute of Tropical Agriculture. Additional details of the research can be found in the parent CRIS project 5450-51000-035-00D.

The objective of this project is to evaluate the bioavailability to humans of iron from two strains of Nigerian corn, and to demonstrate the enhancement of iron bioavailability by increasing the ascorbic acid content of a Nigerian corn-based meal. The project may validate previous testing of the same corn varieties using an in vitro, Caco-2 cell bioavailability method.

This year the study was planned in detail, and reviewed and approved by the University of North Dakota human studies institutional review board, the UND radioactive drug committee, and the USDA radiological safety office. Twenty-seven women have been recruited into and have started participation in the study. The study will be completed within the coming year, including data analysis and manuscript preparation.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/23/2005

Project Number: 5450-51000-035-24R **Accession:** 0409137 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: MARY J KRETSCH
Start Date: 11/16/2004 **Term Date:** 05/31/2006
National Programs: 107 N Human Nutrition
Title: BEANS AND COLON HEALTH IN HUMANS
Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No
Agreement Number: 05-5450-5-0403
Organization Name: MICHIGAN STATE UNIVERSITY

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

Consumption of beans may enhance fermentation in the lower bowel in a manner that helps to inhibit colon cancer, however there is very little experimental evidence in humans to support this hypothesis. A human study was initiated that fed 80 male and female volunteers 200g of beans/day. One-half of the volunteers were healthy adults and one-half had signs and symptoms of metabolic syndrome X. Feces were collected from each subject and used to inoculate an in-vitro fermentation system that was later analyzed for volatile fatty acids. Analyses of the collected samples are ongoing.

4b. List other significant accomplishments, if any.

None

4c. List any significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under a reimbursable agreement number 05-5450-5-0403 between ARS and Beans for Health. Additional details of this research can be found in the report for the parent CRIS 5450-51000-035-00D.

Scientific Publications: **Log 115:**

Approved: CHANDLER LAURENCE D **Date:** 10/03/2005

Project Number: 5450-51000-035-25T **Accession:** 0409464 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH

Start Date: 04/01/2005 **Term Date:** 07/31/2006

National Programs: 107 N Human Nutrition

Title: MICRO FOUNDATIONS OF HEALTH AND ECONOMIC DEVELOPMENT: THE IMPACT OF IRON SUPPLEMENTATION ON SOCIAL AND ECONOMIC PROSPERITY IN INDONESIA

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 05-5450-5-0415

Organization Name: RAND CORPORATION

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

None

4b. List other significant accomplishments, if any.

None

4c. List any significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under trust agreement 58-5450-5-415 with ARS and Rand Corporation. Additional details of research can be found in the report for the parent CRIS project 5450-51000-035-00D.

The objective of this project is to provide biochemical assessment of iron and nutrient status, through analysis and assessment of approximately 1200 blood samples, to help assess the impact of iron supplementation on family economic status in Indonesia. This year the collaboration was planned and documented, human studies approvals were obtained, and the blood samples were analyzed. In the coming year, we will participate in interpretation and reporting of the data, as it relates to additional data collected by the collaborators at UCLA and the RAND corporation.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 09/23/2005

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: DAVID M KLURFELD
Start Date: 07/21/2004 **Term Date:** 04/30/2009
National Programs: 107 N Human Nutrition
Title: ROLE OF DIETARY SELENIUM ON GENE EXPRESSION, CELL CYCLE AND MOLECULAR MECHANISMS IN CANCER RISK
Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?**

Improving the diet by increasing the consumption of whole grains, fruits and vegetables may decrease the incidence of cancer by 30-40%. Although fiber, vitamins and phytochemicals have received the most attention as chemopreventive components of a diet rich in grains, fruit and vegetables, minerals also may be important chemopreventive components. For example, human epidemiologic and supplementation studies, as well as extensive animal studies, have shown the efficacy of selenium in cancer prevention. Food contains different chemical forms of selenium as well as other dietary constituents which will influence the chemopreventive effect of selenium. Furthermore, recent studies suggest that dietary copper protects against colon cancer in several animal models. Other dietary minerals may be beneficial but their role in cancer prevention has not been thoroughly investigated. Mammary, colon and prostate cancers are the main types of cancer which are influenced by dietary factors. A key to understanding the relationship between optimal mineral intake and cancer is determining the effects of mineral intake on cellular processes such as gene expression, oxidative stress, apoptosis and signal transduction. Studies are and will be conducted to determine whether mineral elements such as selenium, copper, and zinc affect biomarkers of carcinogenesis, including carcinogen-induced aberrant crypt formation (a preneoplastic lesion for colon cancer), carcinogen-DNA adduct formation, oxidative status, selenoprotein and detoxifying enzyme activities, DNA methylation and tumor development. Min (multiple intestinal neoplasia) mice will be used to study the effects of trace minerals on the pathogenesis of intestinal cancer in a genetic model for cancer susceptibility. These mice contain a mutation in the murine homolog of the human APC gene and develop spontaneous tumors throughout the intestine. Several observations implicate a role for altered DNA methylation in cancer pathogenesis: the global level of DNA methylation is generally lower but there is gene specific hypermethylation and DNA methyltransferase activity is usually higher in tumor cells than in normal cells. Global DNA methylation, gene specific DNA methylation, methyl metabolism and DNA methyltransferase activity will be evaluated in colon-derived human cells cultured in medium containing different chemical forms of selenium and different concentrations of folate, iron, or zinc and in animals fed diets containing different amounts of selenium, folate, iron or zinc. To determine the mechanisms for the chemopreventive effects of selenium and copper against colon cancer, gene specific macroarrays will be utilized and the effects of copper and selenium on signal transduction pathways for apoptosis and regulation of the cell cycle will be examined in cultured cells. The biological activity of zinc transcription factors will be studied using electrophoretic mobility shift assays or reporter gene constructs. Controlled human feeding studies and/or supplementation studies have and will be conducted to determine whether trace minerals shown to

affect carcinogenesis in animal and cell culture models affect cancer susceptibility in humans. Humans will be fed different diets and fecal water will be analyzed for cytotoxicity, apoptosis, genotoxicity, free radical production and alkaline phosphatase activity. Lymphocytes will be analyzed for DNA methylation, expression of cancer related proteins and measures of oxidative stress/status. Serum from animals or humans fed different concentrations of trace minerals will be used in cell culture systems to investigate cancer susceptibility.

Cancer is the second leading cause of death in the United States. It has been estimated that the cost for the treatment and care of this disease is approaching $200 billion per year. In addition to the economic impact, the development of cancer may prevent many from enjoying life to its fullest. It is believed that diet is the single greatest contributor to human cancer, possibly accounting for 30-40% of the disease. Dietary excesses, deficiencies and imbalances in trace mineral intake are factors that can affect cancer susceptibility. Thus, providing information about requirements and factors that affect those requirements of mineral elements should result in policies and programs that improve intakes of these nutrients that will result in a healthier population, decrease the burden of chronic disease, enhance the quality of life, and diminish health care expenditures.

This research is related to National Program 107, Human Nutrition. The research addresses Performance Goal 3.1.1 of the National Program Action Plan: Human Nutrition Requirements. This research is relevant to Component 1: Nutrient Requirements because one of the priority objectives is to adapt current methods or develop new methods to identify specific disease preventing bioactive dietary factors and elucidate their mechanisms of action. Another priority objective is to use the biomarkers as screening tools to identify the specific bioactive factor(s) responsible for the effects. This research is also relevant to Component 2: Diet, Genetics, Lifestyle and the Prevention of Obesity and Disease. This research will identify the nutrient-relevant influences on gene expression that have consequences on human health and disease.

Several trace minerals have been demonstrated to reduce the risk of developing several types of cancer, but the mechanism by which this occurs is unknown. These studies will address this problem, and thus are of interest to health professionals and policy makers. Understanding the mechanism by which trace elements inhibit cancer has the potential to impact recommendations of how much of the dietary trace mineral should be consumed daily; this in turn has the potential to impact how the medical establishment approaches cancer prevention and how the food industry prepares and/or fortifies specific foods.

2. **List the milestones (indicators of progress) from your Project Plan.**

Year 1 (FY 2005)

Initiate and optimize the HT 29 cell culture studies to determine whether cellular selenium status regulates the cyclin-dependent kinase pathway through the c-Myc gene (1.1).

Initiate and optimize experiments to determine whether methylselenol causes colon cell cycle arrest and induces differential gene expression in the cyclin-dependent kinase pathway (1.3).

Develop constructs and conduct experiments to determine whether thioredoxin reductase (TR) is regulated by selenium availability (dose and chemical form) as well as by ARE inducers and by oxidative stress (2.1 & 2.2).

Determine whether phytochemicals in broccoli act synergistically with selenium in increasing TR activity (2.2).

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Complete the animal portion of the experiment to determine the effect of form and concentration of selenium on methionine sulfoxide activity and expression. Initiate metabolite and enzyme assays (2.6).

Complete animal portion of experiment to study the interaction between selenium and folate. Initiate metabolite and enzyme assays. Begin restriction length genome scanning (RLGS) on select samples (3.3).

Complete and report RLGS on initial (pilot) Ames dwarf mouse study (pre-3.2).

Year 2 (FY 2006)

Finish data collection on the HT 29 cell culture studies designed to determine whether cellular selenium status regulates the cyclin-dependent kinase pathway through the c-Myc gene (1.1).

Finish data collection on the experiments designed to determine whether methylselenol causes colon cell cycle arrest and induces differential gene expression in the cyclin-dependent kinase pathway (1.3).

Perform bioinformatics search/study to understand gene data derived in the above cell culture experiments (1.1 &1.3).

Prepare extracts from plants (other than broccoli) and conduct the studies to determine other compounds that transcriptionally upregulate the TR ARE independent of selenium (2.3).

Complete analyses and report results of hypotheses 2.2-2.3.

Complete analyses and report results from the experiment designed to determine the effect of form and concentration of selenium on methionine sulfoxide activity and expression (2.6).

Complete enzyme and metabolite analysis (including RLGS) from experiment designed to study the interaction between selenium and folate (3.1).

Initiate RLGS on Ames dwarf mice and age-matched wild type controls (3.2).

Year 3 (FY 2007)

Optimize the colon cell culture conditions and start a study to determine how cellular selenium status regulates the mitogen-activated protein kinase pathway (1.2).

Optimize the colon cell culture conditions and start a study to test the hypothesis that selenium-induced apoptotic signaling is different in normal versus transformed cells (1.4).

Determine whether knockdown of TR will have minimal functional consequence for the cell because related ARE-regulated antioxidant systems will be compensatorly upregulated (2.4).

Initiate preliminary experiments to test the hypothesis that simultaneous knockdown of TR and a second ARE-regulated protein (ferritin) will severely damage the ARE-regulated antioxidant network and result in severe cellular damage (2.5).

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2005

Complete methylation assays and gene identification from RLGS in the selenium-folate and Ames dwarf mice studies (3.1 & 3.2).

Initiate RLGS study in Ames mice fed various forms of selenium (3.3).

Year 4 (FY 2008)

Finish cell culture experiments designed to determine how cellular selenium status regulates the mitogen-activated protein kinase pathway (1.2).

Finish cell culture experiments designed to test the hypothesis that selenium-induced apoptotic signaling is different in normal versus transformed cells (1.4).

Initiate studies to determine the role of differentially expressed genes, as discovered in previous cell culture studies, in mediating the anti-tumorigenic effect of selenium (1.5).

Complete the experiments designed to determine the effect of simultaneous knockdown of TR and a second ARE-regulated protein (ferritin) (2.5).

Complete the studies designed to determine whether knockdown of TR will have minimal functional consequence for the cell because related ARE-regulated antioxidant systems will be compensatorly upregulated (2.4).

Complete analyses from the Ames dwarf mouse selenium study (3.3).

Initiate Ames dwarf aberrant crypt study (contingency).

Determine whether there is a relationship between methionine sulfoxide activity and/or expression and aberrant crypt formation in and aberrant crypt model (contingency for 2.6).

Year 5 (FY 2009)

Finish data collection and bioinformatics work on experiments designed to determine the role of differentially expressed genes, as discovered in previous cell culture studies, in mediating the anti-tumorigenic effect of selenium (1.5).

If resources are available, initiate experiments to determine the effect of other dietary antioxidants (other than sulforaphanes and selenium) in ARE-protein knockdown cell models.

Complete the Ames dwarf aberrant crypt study and related methionine sulfoxide studies.

3a. List the milestones that were scheduled to be addressed in FY 2005. For each milestone, indicate the status: fully met, substantially met, or not met. If not met, why.

1. Initiate and optimize the HT 29 cell culture studies to determine whether cellular selenium status regulates the cyclin-dependent kinase pathway through the c-Myc gene (1.1)

   Milestone Fully Met

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2005

3a. 2. Initiate and optimize experiments to determine whether methylselenol causes colon cell cycle arrest and induces differential gene expression in the cyclin-dependent kinase pathway (1.3).

Milestone Fully Met

3. Develop constructs and conduct experiments to determine whether thioredoxin reductase (TR) is regulated by selenium availability (dose and chemical form) as well as by ARE inducers and by oxidative stress (2.1 & 2.2).

Milestone Fully Met

4. Determine whether phytochemicals in broccoli act synergistically with selenium in increasing TR activity (2.2).

Milestone Fully Met

5. Complete the animal portion of the experiment to determine the effect of form and concentration of selenium on methionine sulfoxide activity and expression. Initiate metabolite and enzyme assays (2.6).

Milestone Fully Met

6. Complete animal portion of experiment to study the interaction between selenium and folate. Initiate metabolite and enzyme assays. Begin restriction length genome scanning (RLGS) on select samples (3.3).

Milestone Fully Met

7. Complete and report RLGS on initial (pilot) Ames dwarf mouse study (pre-3.2).

Milestone Substantially Met

3b. List the milestones that you expect to address over the next 3 years (FY 2006, 2007, and 2008). What do you expect to accomplish, year by year, over the next 3 years under each milestone?

FY 2006

Finish data collection on the HT 29 cell culture studies designed to determine whether cellular selenium status regulates the cyclin-dependent kinase pathway through the c-Myc gene (1.1). IMPACT: This study will provide information on how the chemical form of selenium affects cellular antioxidant capability in colon cells.

Finish data collection on the experiments designed to determine whether methylselenol causes colon cell cycle arrest and induces differential gene expression in the cyclin-dependent kinase pathway (1.3). IMPACT: Completion of this study will provide new information on how methylselenol, the putative anticancer form of selenium, affects gene expression as related to anticancer effects in colon tissue.

Perform bioinformatics search/study to understand gene data derived in the above cell culture experiments (1.1 & 1.3). IMPACT: The bioinformatics work will help us determine potential candidate genes that could be used as biomarkers for selenium's chemopreventive actions.

Prepare extracts from plants (other than broccoli) and conduct the studies to determine other compounds that transcriptionally upregulate the TR ARE independent of selenium (2.3). IMPACT: This study will demonstrate the possibility that some of the cancer-preventive aspects of vegetables are mediated by plant phytochemicals activating important antioxidant enzymes.

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2005

Complete analyses and report results from the experiment designed to determine the effect of form and concentration of selenium on methionine sulfoxide activity and expression (2.6). IMPACT: This study will provide information on how dietary selenium affects the activity and expression of the methionine sulfoxides and thus the ability of these enzymes to repair oxidized methionine in proteins.

Complete enzyme and metabolite analysis (including RLGS) from experiment designed to study the interaction between selenium and folate (3.1). IMPACT: Completion of this study will provide new information on how the interaction between dietary selenium and folate affects DNA methylation; this study may result in developing a panel of genes that could be used as biomarkers for selenium's chemopreventive actions.

Initiate RLGS on Ames dwarf mice and age-matched wild type controls (3.2). IMPACT: This study, when completed, will help determine whether the unique methionine metabolism in the Ames dwarf plays a role in the very low cancer rate found in these mice.

FY 2007

Optimize the colon cell culture conditions and start a study to determine how cellular selenium status regulates the mitogen-activated protein kinase pathway (1.2). IMPACT: This study, when completed, will help to determine how selenium affects the expression of cancer genes and thus will help us determine the mechanism of action of selenium.

Optimize the colon cell culture conditions and start a study to test the hypothesis that selenium-induced apoptotic signaling is different in normal versus transformed cells (1.4). IMPACT: This study, when completed, will help to determine whether selenium differentially affects tumor versus normal cells. Thus, if they are affected differently, we will have a better understanding of the mechanism of selenium's anticancer effect in vivo.

Determine whether knockdown of TR will have minimal functional consequence for the cell because related ARE-regulated antioxidant systems will be compensatorly upregulated (2.4). IMPACT: This study will demonstrate that antioxidant protection in a cell is conferred by multiple redundant systems, and knocking out only one system does not result in severe damage to the cell.

Initiate preliminary experiments to test the hypothesis that simultaneous knockdown of TR and a second ARE-regulated protein (ferritin) will severely damage the ARE-regulated antioxidant network and result in severe cellular damage (2.5). IMPACT: This study will demonstrate that although loss of a single component of the antioxidant network will probably result in severe damage to the cell, loss of multiple components of the system will result in severe metabolic abnormalities. This preliminary study will be to determine which components of the system that contain an ARE and can be knocked down by siRNA have the most influence on oxidative status.

Complete methylation assays and gene identification from RLGS in the selenium-folate and Ames dwarf mice studies (3.1 & 3.2). IMPACT: These studies will help determine whether alteration in gene expression by means of DNA methylation is a mechanism though which selenium is anticarcinogenic and whether the unique methionine metabolism in the Ames dwarf plays a role in the very low cancer rate found in these mice.

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Initiate RLGS study in Ames mice fed various forms of selenium (3.3). IMPACT: This study, when completed, will help determine whether selenium acts synergistically in the Ames dwarf mouse to further reduce the incidence of cancer; the study will also be used to determine whether selenium affects methionine metabolism similar to the alteration of methionine metabolism in the Ames dwarf.

FY 2008

Finish cell culture experiments designed to determine how cellular selenium status regulates the mitogen-activated protein kinase pathway (1.2). IMPACT: This study, when completed, will help to determine how selenium affects the expression of cancer genes and thus will help us determine the mechanism of action of selenium.

Finish cell culture experiments designed to test the hypothesis that selenium-induced apoptotic signaling is different in normal versus transformed cells (1.4). IMPACT: This study will help to determine whether selenium differentially affects tumor versus normal cells. Thus, if they are affected differently, we will have a better understanding of the mechanism of selenium's anticancer effect in vivo.

Initiate studies to determine the role of differentially expressed genes, as discovered in previous cell culture studies, in mediating the anti-tumorigenic effect of selenium (1.5). IMPACT: This study, when completed, will help confirm some of our in vitro findings in the in vivo model. Thus, it may result in finding biomarker candidate genes for selenium's chemopreventive actions.

Complete the experiments designed to determine the effect of simultaneous knockdown of TR and a second ARE-regulated protein (ferritin) (2.5). IMPACT: This study will utilize procedures developed FY 2007 and will provide definitive evidence that loss of multiple components of the system will result in severe metabolic abnormalities.

Complete the studies designed to determine whether knockdown of TR will have minimal functional consequence for the cell because related ARE-regulated antioxidant systems will be compensatorly upregulated (2.4). IMPACT: This study will use tools such as gene arrays to demonstrate that the loss of one component of the system is compensated for by upregulation of many other components of the system.

Complete analyses from the Ames dwarf mouse selenium study (3.3). IMPACT: This study will help determine whether selenium acts synergistically in the Ames dwarf mouse to further reduce the incidence of cancer; the study will also be used to determine whether selenium affects methionine metabolism similar to the alteration of methionine metabolism in the Ames dwarf.

Determine whether there is a relationship between methionine sulfoxide activity and/or expression and aberrant crypt formation in and aberrant crypt model (contingency for 2.6). IMPACT: This study, when completed will help determine whether the mode of action of selenium anticarcinogenicity is through methionine sulfoxide activity and/or expression.

4a. What was the single most significant accomplishment this past year?

Butyrate treatment inhibits the migration and invasion potential of tumor cells: Butyrate, a product of the bacterial fermentation of dietary fiber, has been hypothesized to be directly related to the lower risk of colon cancer. It is important to test and to characterize the invasive ability of tumor cells as affected by long exposure to low concentrations of butyrate. Our results demonstrate that prolonged and low-dose butyrate treatment (0.5 mmol/L butyrate, similar to

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2005

moderate fiber diet) inhibits pro-MMP-2 activation and tumor cell migration/invasion potential. Our data provide a possible mechanism for butyrate's anticarcinogenic properties. IMPACT: These data support the health claim of a high-fiber diet and provide a mechanism to link fiber and decreased cancer risk.

4b. List other significant accomplishments, if any.

Thioredoxin reductase is regulated by sulforaphane: Thioredoxin reductase, a selenium-requiring protein, is transcriptionally regulated by sulforaphane from broccoli. Broccoli contains many other compounds that may inhibit cancer, and some of this inhibition may be mediated by turning on ARE containing enzymes such thioredoxin reductase. This hypothesis was investigated by adding compounds known to be in broccoli to cells in culture containing a reporter gene construct of thioredoxin reductase. Although ascorbic acid resulted in modest induction, the results convincingly demonstrated that sulforaphane was responsible for almost all of the ARE-mediated activation of thioredoxin reductase. IMPACT: This research provides a plausible mechanism to explain how broccoli consumption may inhibit cancer. Furthermore, this study provides a tool that can be used to screen various compounds to determine their cancer-fighting ability.

Glycine N-methyltransferase, a tumor susceptibility gene, is decreased in selenium deficiency: Determined that the activity of the enzyme glycine N-methyltransferase (GNMT) is decreased by selenium deprivation. GNMT, which has been shown to be a tumor susceptibility gene, is a regulator of tissue S-adenosylmethionine concentration, and in liver, is a major folate binding protein. Thus, GNMT may induce changes in tissue folate status resulting in chromosome breakage or abnormal DNA methylation. Second, GNMT is an enzyme participating in detoxification. In addition, GNMT may have a protective effect against the exposure to carcinogens by decreasing DNA adduct formation. Thus, the decrease in this enzyme may explain some of the effects of selenium deficiency. IMPACT: This study affirms the importance of the interaction between dietary selenium and folic acid and suggests that alterations in selenium status may affect folate status and vice versa. This may prove most important in the nutrition of those humans who may have low folate and low selenium status. That is, supplementation of one without the other may be more detrimental than beneficial.

Transsulfuration is markedly enhanced in the long-lived Ames dwarf mouse: Showed by using tracer studies that transsulfuration is markedly enhanced in the Ames dwarf mouse. The Ames dwarf mouse lives substantially longer and has a lower incidence of cancer that the wild type. This tracer study, along with real-time RT PCR findings of genes associated with methionine metabolism, provides a plausible mechanism for the increased glutathione found in the dwarf mice. IMPACT: This supports the hypothesis that these mice have enhanced oxidative defense capabilities. This study gives insight to what mechanisms in animals are important in aging and in decreasing the risk of cancer; similar mechanisms (and how nutrition affects them) could then be studied in humans.

4c. List any significant activities that support special target populations.

None.

4d. Progress report.

None.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

This research is directly related to the ARS National Human Nutrition Research Plan

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107, Component 2: Diet, Genetics, Lifestyle and the Prevention of Disease, and directly contributes to the accomplishmnet of ARS Strategic Plan Goal #4, Improve the Nation's Nutrition and Health.

[This report is the first for this CRIS project (certified in 2005), thus the accomplishments for the life of the project are incorporated in sections 4a and 4b.]

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**

   Information on how trace elements (specifically selenium) affect mechanisms related to carcinogenesis was presented at numerous workshops and scientific meetings.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below).**

   Two presentations were made in the fourth quarter FY04: A Grand Forks Herald article entitled "Plant-based diet may be healthiest choice" and a talk to the Asian Horticultural Society entitled "Efficacy of plant-based compounds for cancer reduction."

Scientific Publications: **Log 115:**

1. Brown-Borg, H.M., Radoczy, S.G., Uthus, E.O. 2004. Growth hormone alters methionine and glutathione metabolism in Ames dwarf mice. Mechanisms of Ageing and Development 126:389-398. **0000168664**
2. Davis, C.D., Uthus, E.O. 2004. DNA methylation, cancer susceptibility, and nutrient interactions. Experimental Biology and Medicine. 229:988-995. **0000183301**
3. Uthus, E.O., Davis, C.D. 2005. Dietary arsenic affects dimethylhydrazine-induced aberrant crypt formation and hepatic global DNA methylation and DNA methyltransferase activity in rats. Biological Trace Element Research. 103:133-145. **0000159067**
4. Zeng, H., Briske Anderson, M.J. 2005. Prolonged butyrate treatment inhibits the migration and invasion of potential HT1080 tumor cells. Journal of Nutrition. 135:291-295. **0000169594**
5. Zeng, H., Uthus, E.O., Combs, G.F. 2005. Mechanistic aspects of the interaction between selenium and arsenic. Inorganic Biochemistry. 99:1269-74. **0000176434**
6. Combs, G.F. 2005. Current evidence and research needs to support a health claim for selenium and cancer prevention. Journal of Nutrition. 135:343-347. **0000166681**
7. Combs, Jr., G.F. 2004. Status of Selenium in Prostate Cancer Prevention. British Journal of Cancer. 91:195-199. **0000162566**
8. Combs Jr., G.F., Patterson, B., Brindak, M., Midthune, D., Taylor, P., Veillon, C., Patterson, K., Hill, D., Levander, O.A. 2005. Thyroid hormone levels in subjects supplemented with oral selenomethionine [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1016. **0000171690**
9. Patterson, B., Wastney, M.E., Combs Jr., G.F., Brindak, M., Patterson, K.Y., Veillon, C., Taylor, P., Levander, O.A. 2005. Se metabolism in humans is altered by long-term supplementation [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1015. **0000171726**

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10. Uthus, E.O., Ross, S., Davis, C. 2005. Differential effects of dietary selenium (Se) and folate on methyl metabolism in liver and colon or rats [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1013. **0000171155**

11. Zeng, H., Briske-Anderson, M. 2005. Inhibitory effect of prolonged-butyrate treatment on migration and invasion of HT1080 tumor cells [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1693. **0000171283**

Approved: CHANDLER LAURENCE D **Date:** 09/20/2005

Project Number: 5450-51000-036-01R **Accession:** 0406009 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: DAVID M KLURFELD

Start Date: 07/15/2002 **Term Date:** 06/30/2007

National Programs: 107 N Human Nutrition

Title: DETERMINATION OF NUTRIENT EFFECTS ON CANCER SUSCEPTIBILITY ON EPIGENETIC PROCESSES IN ANIMAL MODELS

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 02-5450-2-0217

Organization Name: **NATIONAL CANCER INSTITUTE, DEPARTMENT OF HEALTH AND HUMAN SERVICES**

Progress and Outcomes:

4d. Progress report.

This report serves to document research conducted under a reimbursable interagency agreement #02-5450-2-0217 with the National Cancer Institute. This work supports that done in 5450-51000-031-00D, Role of Selenium on Gene Expression, Cell Cycle and Molecular Mechanisms in Cancer Risk.

The purpose of this agreement is to examine the interactive effects of dietary selenium and folate on cancer susceptibility, global and gene specific DNA methylation, DNA methyltransferase activity, and one-carbon metabolism using animal models. This research indicates the possibility that dietary selenium and folic acid prevent cancer-causing genes (oncogenes) from being turned on and/or cancer-suppressing genes (tumor suppressor genes) from being turned off. Both selenium and folic acid can affect the addition of a single carbon molecule (methylation) to specific areas on DNA. The amount and pattern of methylation of DNA can result in genes being turned on or off (and thus affect cancer susceptibility). A preliminary study was conducted to determine the feasibility of using a technique, called restriction landmark genomic scanning (RLGS), to scan thousands of genes for differences in DNA methylation in rats fed graded amounts of dietary selenium (as inorganic selenite) and the vitamin folic acid. The results indicate that dietary selenium and folic acid can affect specific DNA methylation patterns. This is important as it provides a mechanism in which diet can affect the cancer process. RLGS for the preliminary study was run at OSU. The method has now been successfully transferred to the GFHNRC. An animal study has been completed and the tissues (liver initially) will be analyzed by RLGS. Tissues from this experiment will also be used in gene array analysis as another screening tool to determine which genes are affected by selenium and folic acid. The tissues (liver, colonic mucosa, prostate, and lung) have been sent to NCI for gene array analysis. The experiment was designed to test dose and form of selenium (selenite, selenomethionine, and Se-methylselenocysteine) to determine the best form of selenium for cancer prevention.

We expect that numerous genes, as determined by microarray analysis, will be affected by dietary selenite and/or folic acid. Real-time RT PCR will be used to validate the microarrays (specific genes only). We also expect the several genes will be found to be affected by methylation, as determined by RLGS. Enzyme and metabolite analyses will help us ascertain possible mechanisms or outcome of the differentially methylated gene(s) (and hence reduced or increased expression). The

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outcome of this research will assist in the elucidation of mechanisms by which specific nutritional factors - selenium and folate - influence DNA methylation, or other novel processes, as well as increase our understanding of these processes and dietary factors in relation to cancer prevention. It is expected that the results will confirm and expand our previous findings that indicted that selenium supplemented at supranutritional concentrations is beneficial when folic acid is present in the diet but detrimental when folic acid is inadequate.

Progress was made on establishing the methodology to use the Big Blue rodent model to evaluate spontaneous mutation rates as affected by diet. This model uses transgenic rats that carry a chromosomally integrated lambda bacteriophage containing E. coli lacI gene as a target for mutation. Lambda phage are rescued by in vitro packaging and screened for mutations. This model will be used to determine whether dietary selenium (form and dose) affect spontaneous mutation rates. A pilot study to develop the required procedures was successfully conducted. A full scale study is now being planned.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 08/31/2005

FINAL PROGRESS REPORTS

OF

TERMINATED CRIS WORK UNITS

NUTRITIONAL DETERMINANTS OF HEALTH

MANAGEMENT UNIT

5450-010-00

Project Number: 5450-51000-034-00D **Accession:** 0405069 **FY:** 2005
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH

Start Date: 02/22/2002 **Term Date:** 07/31/2005

National Programs: 107 N Human Nutrition

Title: MINERAL INTAKES FOR OPTIMAL BONE AND JOINT DEVELOPMENT AND HEALTH

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** Yes
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?**

Osteoporosis is a prevalent and costly bone disease that causes at least one bone fracture in one out of every two women and one out of eight men in their lifetimes. The annual cost of healthcare related to osteoporosis is estimated at $14 billion. In the elderly, hip fractures are associated with mortality in up to 20% of the cases. Osteoporosis is a multifactorial disease process directed by several factors. These risk factors include environment (i.e., nutrition, physical activity), genetics, endogenous hormones (estrogen and androgen deficiencies), and age. Behavior modification and pharmacologic intervention (e.g., hormone replacement therapy [HRT]) are the common complementary approaches used to prevent bone loss in asymptomatic women. However, only 12-20% of US postmenopausal women currently use HRT. Thus, nutrition is, arguably, one of the most important modifiable factors and represents a primary approach in prevention of this debilitating disease. For this reason, all significant nutritional factors must be identified that maximize peak bone mass during development and maintain bone mass and strength during aging.

There is consensus that adequate calcium intake continuous from childhood is critical for the formation and maintenance of a healthy skeleton. Thus, dietary calcium supplementation is considered as both a treatment and prophylaxis for osteoporosis. However, there is no current recommended daily allowance (RDA) for this important nutrient because experimental data are not available to assess adequately the physiological adaptation to changes in calcium intake over time. Furthermore, calcium metabolism is known to be modified by many other dietary factors including dietary protein sources (vegetable and animal), trace minerals (e.g., copper, zinc, and boron), and prebiotics (e.g., inulin). However, to date, the interaction of these factors with calcium is at best ill-defined. Therefore, this project had two specific goals: 1) titration of the calcium intake needed to optimize bone health and prevent bone loss in postmenopausal women and 2) determination of the roles of key dietary factors (protein, selected trace elements, and prebiotics) on the utilization of dietary calcium.

Animal and human experiments were conducted with the goal of establishing the calcium requirement and characterizing the modifying roles of animal proteins (in meat and milk), and zinc, copper, magnesium, and boron (as trace elements) on that requirement. Postmenopausal women and appropriate animal models (e.g., ovariectomized female rats) consumed varying amounts (low, adequate, and/or supranutritional) of calcium and the identified dietary factors while all other components of the diet remained constant. The response of the animals and humans to the dietary manipulations was ascertained by evaluating appropriate biochemical, physiological, and anatomical variables.

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The research undertaken fell under National Program 107, Human Nutrition, and addressed goal 3.1.1 (Human Nutrition Requirements). The challenge of this component is to identify essential nutrients, determine their effects on reproduction, development, function and longevity, and to provide information that will be used to develop standards to optimize human health, well-being, and genetic potential throughout the life cycle. All priority objectives, especially mechanism of action, biomarkers, function and performance, and nutrient interactions apply to the research program. Outcomes of the research was knowledge that will facilitate establishment of an RDA for calcium with due consideration of the dietary factors that have significant potential to modify that requirement.

2. **List the milestones (indicators of progress) from your Project Plan.**

Because this project plan was under development, no formal milestones were in place. The review process was completed and this project was replaced by another project plan (5450-51000-039-00D, Mineral Intakes for Optimal Bone Development and Health) that continues work in a similar area and was initiated 08/01/2005. Milestones from the new approved plan are listed below:

FY 2005:
Enroll subjects and conduct study on estimating the Ca requirement by titration
Conduct the first of a three year study on bone health and copper and zinc supplementation
Complete in utero phase of boron essentiality study
Initiate growth phase of three study of boron essentiality

FY 2006:
Complete data analyses from study on estimating the Ca requirement by titration
Enroll subjects and conduct study on bone health during weight loss
Conduct the second of a three year study on bone health and copper and zinc supplementation
Report findings from in utero phase of boron essentiality study
Conduct the second of a three year study of boron essentiality
Conduct study on whether dietary boron affects calcium absorption
Enroll subjects and conduct study on whether dietary inulin affects calcium absorption

FY 2007:
Report on study on estimating the Ca requirement by titration
Complete analyses from study on bone health during weight loss
Conduct the third of a three year study on bone health and copper and zinc supplementation
Conduct the third of a three year study of boron essentiality
Conduct post-growth phase of boron essentiality study
Enroll subjects and conduct study on whether magnesium status affects calcium retention and bone resorption
Complete analyses from study on whether dietary inulin affects calcium absorption

FY 2008:
Report on study of whether bone health is affected by weight loss
Complete analyses on study on bone health and copper and zinc supplementation
Report findings from growth phase of boron essentiality study
Report findings from post-growth phase of boron essentiality study
Complete analyses on study whether magnesium status affects calcium retention and bone resorption

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Report findings from dietary inulin and calcium absorption study

3a. List the milestones that were scheduled to be addressed in FY 2005. For each milestone, indicate the status: fully met, substantially met, or not met. If not met, why.

1. Conduct the second of a three year study to determine whether dietary copper and/or zinc enhance the ability of calcium to attenuate bone loss

   Milestone Fully Met

2. Complete in utero phase of boron essentiality study

   Milestone Fully Met

3. Initiate growth phase of boron essentiality study

   Milestone Fully Met

4. Enroll recruitment of subjects and conduct study on estimating the Ca requirement by titration

   Milestone Not Met

   Reason not met: Critical SY Vacancy

3b. List the milestones that you expect to address over the next 3 years (FY 2006, 2007, and 2008). What do you expect to accomplish, year by year, over the next 3 years under each milestone?

Development of this project was completed and replaced by another project plan (5450-51000-039-00D, Mineral Intakes for Optimal Bone Development and Health) that continues work in a similar area and was initiated 08/01/2005. Milestones from the new approved plan are listed.

FY 2006:
Complete data analyses from study on estimating the Ca requirement by titration.
A study to determine the amount of dietary calcium needed to maximize calcium retention and minimize bone resorption in postmenopausal women has been delayed because of a critical SY vacancy. Because existing experimental data are not adequate to assess physiological adaptation(s) to changes in calcium intake over time, the information from this study will be useful to Dietary Reference Committees in establishing a Recommended Daily Allowance for calcium in postmenopausal women.

Enroll subjects and conduct study on bone health during weight loss.
A study will be planned and initiated to determine whether a balanced, high protein diet, one providing moderate amounts of carbohydrate, is adequate to maintain calcium homeostasis. Because changes in dietary patterns are needed to combat the American epidemic of obesity, rigorous tests of weight loss strategies (consumption of high protein diets) on calcium retention are needed. The tests will be useful in helping develop guidelines that will protect against bone loss.

Conduct the second of a three year study on bone health and copper and zinc supplementation
A three year study is underway to determine whether dietary supplements of copper and/or zinc enhance the ability of calcium to attenuate bone loss in postmenopausal

women. Because osteoporosis is a multifactorial disease not prevented by adequate calcium and vitamin D nutrition alone, this research will characterize the interactions of calcium with zinc and copper, two elements that show special promise in affecting bone calcification.

Report findings from in utero phase of boron essentiality study.
Findings will be reported from a study to determine whether or not boron affects the quantitative need for calcium and is essential for bone health during the in utero phase of development. Because adequate calcium intake continuous from development is considered critical for the formation and maintenance of a healthy skeleton, this research will characterize some of the interactions of calcium with boron, an element that benefits bone calcification.

Conduct the second of a three year study of boron essentiality during growth.
A three year study is underway to determine whether or not boron affects the quantitative need for calcium and is essential for bone health during the postnatal phase of development. Because adequate calcium intake continuous from development is considered critical for the formation and maintenance of a healthy skeleton, this research will characterize some of the interactions of calcium with boron, an element that benefits bone calcification.

Conduct study on whether dietary boron affects calcium absorption.
A study will be completed on whether dietary boron deprivation impairs calcium absorption especially at marginal calcium intakes. Because osteoporosis is a multifactorial disease not prevented by adequate calcium and vitamin D nutrition alone, this research will characterize the interactions of calcium with boron, an element that benefits bone calcification.

Enroll subjects and conduct study on whether dietary inulin affects calcium absorption.
A study will be conducted with healthy postmenopausal women to determine whether inulin, a prebiotic, sufficiently enhances calcium absorption to be of practical significance for bone health. Because osteoporosis is a multifactorial disease of unknown etiology, this research will characterize specific health effects of inulin, a natural nondigestible carbohydrate with apparent potential to enhance calcium absorption.

FY 2007:
Report on study on estimating the Ca requirement by titration.
A study to determine the amount of dietary calcium needed to maximize calcium retention and minimize bone resorption in postmenopausal women has been delayed because of a critical SY vacancy. Because existing experimental data are not adequate to assess physiological adaptation(s) to changes in calcium intake over time, the information from this study will be useful to Dietary Reference Committees in establishing a Recommended Daily Allowance for calcium in postmenopausal women.

Complete analyses from study on bone health during weight loss.
Data analyses will be completed on a study to determine whether a balanced, high protein diet, one providing moderate amounts of carbohydrate, is adequate to maintain calcium homeostasis. Because changes in dietary patterns are needed to combat the American epidemic of obesity, rigorous tests of weight loss strategies (consumption of high protein diets) on calcium retention are needed. The tests will be useful in helping develop guidelines that will protect against bone loss.

Conduct the third of a three year study on bone health and copper and zinc

supplementation.
A three year study is underway to determine whether dietary supplements of copper and/or zinc enhance the ability of calcium to attenuate bone loss in postmenopausal women. Because osteoporosis is a multifactorial disease not prevented by adequate calcium and vitamin D nutrition alone, this research will characterize the interactions of calcium with zinc and copper, two elements that show special promise in affecting bone calcification.

Conduct the third of a three year study of boron essentiality during growth.
A three year study is underway to determine whether or not boron affects the quantitative need for calcium and is essential for bone health during the postnatal phase of development. Because adequate calcium intake continuous from development is considered critical for the formation and maintenance of a healthy skeleton, this research will characterize some of the interactions of calcium with boron, an element that benefits bone calcification.

Conduct post-growth phase of boron essentiality study.
A study will be completed on whether dietary boron affects the quantitative need for calcium and is essential for bone health during the post-growth phase of the life cycle. Because osteoporosis is a multifactorial disease not prevented by adequate calcium and vitamin D nutrition alone, this research will characterize the interactions of calcium with boron, an element that benefits bone calcification.

Enroll subjects and conduct study on whether magnesium status affects calcium retention and bone resorption.
A study will be planned and initiated to determine whether marginal magnesium status increases bone resorption and changes calcium needs in postmenopausal women. Because osteoporosis is a multifactorial disease not prevented by adequate calcium and vitamin D nutrition alone, this research will characterize the interactions of calcium with magnesium, an element that may be a risk factor for osteoporosis.

Complete analyses from study on whether dietary inulin affects calcium absorption.
Data analyses will be completed from a study conducted with healthy postmenopausal women to determine whether inulin, a prebiotic, sufficiently enhances calcium absorption to be of practical significance for bone health. Because osteoporosis is a multifactorial disease of unknown etiology, this research will characterize specific health effects of inulin, a natural nondigestible carbohydrate with apparent potential to enhance calcium absorption.

FY 2008:

Report on study of whether bone health is affected by weight loss.
Findings will be reported from a study to determine whether a balanced, high protein diet, one providing moderate amounts of carbohydrate, is adequate to maintain calcium homeostasis. Because changes in dietary patterns are needed to combat the American epidemic of obesity, rigorous tests of weight loss strategies (consumption of high protein diets) on calcium retention are needed. The tests will be useful in helping develop guidelines that will protect against bone loss.

Complete analyses on study on bone health and copper and zinc supplementation
Data analysis will be completed from study to determine whether dietary supplements of copper and/or zinc enhance the ability of calcium to attenuate bone loss in postmenopausal women. Because osteoporosis is a multifactorial disease not prevented by adequate calcium and vitamin D nutrition alone, this research will characterize the interactions of calcium with zinc and copper, two elements that

Project Number: 5450-51000-034-00D **Accession:** 0405069 **FY:** 2005

show special promise in affecting bone calcification. Information from this study will be useful to Dietary Reference Committees in establishing a Recommended Daily Allowance for calcium in postmenopausal women.

Report findings from growth phase of boron essentiality study.
Findings will be reported from a study on whether or not boron affects the quantitative need for calcium and is essential for bone health during the postnatal phase of development. Because adequate calcium intake continuous from development is considered critical for the formation and maintenance of a healthy skeleton, this research will characterize some of the interactions of calcium with boron, an element that benefits bone calcification. This information is needed by Dietary References Committees to help establish an adequate intake of boron in adolescents.

Report findings from post-growth phase of boron essentiality study.
Findings will be reported from a study on whether dietary boron affects the quantitative need for calcium and is essential for bone health during the post-growth phase of the life cycle. Because osteoporosis is a multifactorial disease not prevented by adequate calcium and vitamin D nutrition alone, this research will characterize the interactions of calcium with boron, an element that benefits bone calcification. This information is needed by Dietary References Committees to help establish an adequate intake of boron in mature adults.

Complete analyses on study whether magnesium status affects calcium retention and bone resorption.
Data analyses will be completed for a study to determine whether marginal magnesium status increases bone resorption and changes calcium needs in postmenopausal women. Because osteoporosis is a multifactorial disease not prevented by adequate calcium and vitamin D nutrition alone, this research will characterize the interactions of calcium with magnesium, an element that may be a risk factor for osteoporosis. The information is needed by Dietary References Committees to refine the Recommended Daily Allowance for magnesium in postmenopausal women.

Report findings from dietary inulin and calcium absorption study.
Findings will be reported from a study with healthy postmenopausal women to determine whether inulin, a prebiotic, sufficiently enhances calcium absorption to be of practical significance for bone health. Because osteoporosis is a multifactorial disease of unknown etiology, this research will characterize specific health effects of inulin, a natural nondigestible carbohydrate with apparent potential to enhance calcium absorption. This information is needed to determine whether inulin, a prebiotic, sufficiently enhances calcium absorption to be of practical significance for bone health.

4a. What was the single most significant accomplishment this past year?

Boron Reduces Inflammation Signals From Cells Involved in Inflammation:
There is evidence that dietary boron helps control the normal inflammatory process and thereby helps prevent development of bone diseases such as rheumatoid arthritis. Scientists at the Grand Forks Human Nutrition Research Center, in cooperation with scientists at the University of North Dakota, Grand Forks, ND, used immune function cells taken from mice to determine how boron prevents inflammatory disease. The cells were grown in a culture solution that was low in boron or was supplemented with boron. When the cells were stimulated with an agent to produce inflammatory signal molecules, gene expression of those signal molecules was suppressed in those cells supplied with boron. This accomplishment is important because it shows a likely mechanism by which dietary boron keeps the normal inflammatory system in

check.

4b. List other significant accomplishments, if any.

Boron and Fish Oil Act Synergistically to Enhance Bone Strength:
Boron deprivation and a diet with safflower oil as the fat source resulted in long bone strength that was increased synergistically by both boron supplementation and providing fish oil instead of safflower oil in the diet. The increase in bone strength may have been the result of improved bone microarchitecture because boron supplementation increased vertebra trabecular thickness and fish oil increased trabecular bone volume. Some studies show that, compared to diets high in omega-6 polyunsaturated fatty acids (PUFA), diets high in omega-3 PUFA promote bone accretion and strength, but other studies show no marked effects. The inconsistency may be the result of different intakes of another nutrient, such as boron, shown to affect similar functions as the long chain omega-3 PUFA. This accomplishment is important because it shows that the beneficial effects of fish oil or omega-3 fatty acids on bone health may be maximized with a diet containing boron-rich foods (fruits, vegetables and pulses).

High Protein Intake Enhances Calcium Retention From A Low Calcium Diet:
A controlled feeding study with healthy postmenopausal women was conducted to determine whether dietary protein and calcium interact to affect calcium retention and thus bone health. The subjects were fed a series of diets that were low in Ca (600 mg/d) or high in Ca (1500 mg/d) and low protein or high in protein (10% and 20% of energy mostly as meat protein, respectively). The four diets were fed for 7 weeks each in random order. After 3 weeks of equilibration, the menu was labeled with a tracer dose of radioactive calcium to monitor how long the calcium stayed in the body. Regardless of how much calcium was consumed, a high protein diet increased the amount of calcium in the urine. When calcium intake was low, a high protein diet, compared to a low protein diet, enhanced the initial absorption of calcium and also increased calcium retention. As expected, a high calcium intake, compared to a low calcium intake, reduced the fractional amount of calcium retained. A high protein intake also decreased bone resorption, as indicated by the amount of a bone marker found in urine. This accomplishment is important because it shows that, in postmenopausal women, a moderately high protein diet may be beneficial to bone health, especially when calcium intake is low.

Antirachitic Properties of Dietary Calcium:
A 13 mo. long, double-blind, clinical trial was conducted in 1-5 yr. old children at risk to developing rickets in a rickets-endemic part of Bangladesh. Subjects were randomized to four treatments consisting of a milk powder-based dietary supplement providing graded levels of calcium (Ca) with or without multi-vitamins and minerals. When 183 healthy children presenting with no rachitic leg signs but serum alkaline phosphatase activities in the upper decile were re-screened after a 7 mo. pre-trial period, 23 (12.6%) showed rachitic leg signs, suggesting an annual risk of 21.5% in this cohort. Of those still not presenting with leg signs after the pre-trial period and completing 13 mos. of dietary intervention, none showed rachitic leg signs and all showed carpal ossification normal for age after that intervention. At that time, however, 18 subjects (16%) showed modest radiographic knee deformations (<168 degrees) without significant differences between treatment groups. This accomplishment is important because it shows that even very small supplements of calcium (50 mg/d) are useful in supporting normal bone development in this high-risk population.

Project Number: 5450-51000-034-00D **Accession:** 0405069 **FY:** 2005

Dietary Boron Reduces Serum Insulin Concentrations Regardless of Vitamin D Status: There is evidence that dietary boron helps control the normal levels of insulin in blood. Scientists at the Grand Forks Human Nutrition Research Center, in cooperation with scientists at North Dakota State University, Fargo, ND, determined whether dietary boron works through vitamin D to affect serum insulin levels. As expected, dietary boron lowered insulin levels when vitamin D status was adequate. Also, as expected, severe vitamin D deficiency lowered insulin levels in the blood far below the normal range but adding boron to the diet did not change that effect. The results suggest that dietary boron affects blood insulin concentrations independent of vitamin D status. Because dietary boron reduces circulating insulin concentrations while maintaining serum glucose concentrations within the normal range, dietary boron might help prevent pancreatic exhaustion caused by chronic hyperinsulinemia.

4c. List any significant activities that support special target populations.

None

4d. Progress report.

None

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

High Protein Diets May Not Adversely Affect Bone Health in Healthy Postmenopausal Women:
To determine whether animal protein intake is a risk for the development of osteoporosis, a carefully controlled feeding study of several weeks duration was conducted in postmenopausal women at the Grand Forks Human Nutrition Research Center. Findings from this study indicate that a high protein diet (20% of energy mostly provided as meat) may not increase calcium loss and or adversely affect bone health in healthy postmenopausal women. On the contrary, a significant improvement in calcium retention was observed in women with low calcium intake. These findings, which contradict prevalent assumptions about the effects of meat consumption on calcium retention, will improve the quality and nature of the advice given to the public regarding the effects of dietary protein, especially meat protein, on bone health. This accomplishment was conducted under National Program 107 Human Nutrition; ARS Strategic Plan goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining interactions for known classes of nutrients.

High Protein Diets May Be Protective Against Bone Loss:
A study to determine the effects of high-isoflavone soy protein versus beef on calcium retention and biomechanical properties of bones was conducted at the Grand Forks Human Nutrition Research Center in collaboration with the University of North Dakota, Grand Forks, ND. In this study with ovarioectomized rats (estrogen-deficient), the findings indicated that calcium retention was significantly improved when protein intake was high (20%) and it was higher in the animals consuming beef versus soy protein. Serum IGF-1, a growth factor needed for bone health, was also higher in the animals consuming the high protein diets (20% soy or beef). These results are consistent with our findings from human studies and indicate that a high protein diet may be protective against bone loss in postmenopausal women rather than harmful. This accomplishment was conducted under National Program 107 Human Nutrition; ARS Strategic Plan goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining interactions for known classes of nutrients.

Project Number: 5450-51000-034-00D **Accession:** 0405069 **FY:** 2005

Dietary Silicon May Have a Role in Bone Formation:
An experiment was performed at the Grand Forks Human Nutrition Research Center to determine whether silicon deficiency in mature rats would exacerbate bone loss induced by ovariectomy (a model of osteoporosis). Silicon deprivation did not affect calcified bone changes induced by ovariectomy, but independently changed blood markers of bone metabolism and bone composition associated with the organic matrix. The findings suggest that silicon is a bioactive food component that is involved in the proper formation of the organic scaffold upon which calcified bone is formed. This accomplishment was conducted under National Program 107 Human Nutrition; ARS Strategic Plan goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining functions for emerging classes of nutrients.

Boron in Breast Milk May be Under Homeostatic Control:
To determine whether the amount of boron in human milk is regulated, scientists at the Grand Forks Human Nutrition Research Center, in cooperation with scientists at the University of Manitoba, Winnipeg, Canada, and the USDA/ARS Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX, obtained definitive data on the concentration of boron in human milk during the first four months of lactation. Boron milk concentrations were remarkably similar between the separate populations and stable over four months of lactation. This accomplishment is important because it clearly shows that boron concentrations are similar in separate populations and therefore probably under homeostatic control. This accomplishment was conducted under National Program 107 Human Nutrition; ARS Strategic Plan goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining functions for emerging classes of nutrients.

Dietary Boron May Provide Resistance to Rheumatoid Arthritis:
Studies were conducted at the Grand Forks Human Nutrition Research Center in collaboration with the University of North Dakota to characterize further the effect of dietary boron on the onset of collagen-induced arthritis (an animal model of polyarthritis similar to human rheumatoid arthritis). Similar to our previous studies, mice weaned onto low-, adequate-, or luxuriant-boron diets exhibited a 54, 29, and 13% incidence of arthritis respectively. The findings indicate that dietary boron provides resistance to clinical signs in this animal model of rheumatoid arthritis. This accomplishment was conducted under National Program 107 Human Nutrition; ARS Strategic Plan goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining functions for emerging classes of nutrients.

Very High Amounts of Calcium May Compromise Iron Status of Some Individuals:
A study was conducted with growing female rats to determine whether an interaction between calcium and iron affects the biomechanical and biochemical properties of bone. The findings from this study, done in collaboration with the University of North Dakota, indicate that higher than adequate calcium intakes reduced both iron status and bone quality. These findings indicate that very high calcium intakes, as may result from indiscriminate supplementation and fortification of foods with calcium, may compromise the iron status of vulnerable segments of the population without offering additional benefits to bone health. This accomplishment was conducted under National Program 107 Human Nutrition; ARS Strategic Plan goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining interactions for known classes of nutrients.

Consuming Foods High in Magnesium and Nickel May Benefit Bone Health:
A study was conducted to determine whether bone health is impaired by a combined

lack of dietary nickel and magnesium, two minerals generally supplied by the same foods. The combined marginal deficiency of magnesium and nickel resulted in increased prostaglandin E-2 excretion and changed the mineral composition of bone. The findings indicate that consuming foods high in both magnesium and nickel, including leafy green vegetables, legumes, whole grains and nuts, is beneficial to bone health, and that the beneficial effects possibly occur through these minerals influencing the metabolism of lipids to prostaglandins that promote bone formation. This accomplishment was conducted under National Program 107 Human Nutrition; ARS Strategic Plan goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining functions for emerging classes of nutrients.

Full Substitution of Soy Protein for Meat Protein May Reduce Calcium Retention:
A high meat intake is often cited as a risk factor for development of osteoporosis, and soy protein is thought to have a beneficial effect on bone health. However, new reported findings indicate that partial substitution of soy protein (with high isoflavones) for meat protein does not improve calcium retention in postmenopausal women. In a follow-up study with ovarioectomized rats, complete substitution of meat protein by soy protein isolate with high isoflavone content reduced calcium retention by about 30%. Assuming that the findings in rats are applicable to humans, these combined results indicate that, contrary to popular belief, meat protein intake does not adversely affect bones and that while a partial substitution of soy protein for meat protein may not reduce calcium retention, full replacement may adversely affect calcium retention. This accomplishment was conducted under National Program 107 Human Nutrition; ARS Strategic Plan goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to interactions functions for known classes of nutrients.

A Diet Low in Silicon May Impair Bone Mineralization:
A series of experiments with rats performed both in-house and with collaborators at the University of Wisconsin-Stout, Stout, WI, and the University of Buenos Aires have shown that silicon deprivation is detrimental to bone physical characteristics and repair apparently through changing collagen and glycosaminoglycan metabolism during development and maturation of the organic matrix upon which bone mineralization occurs. The findings have resulted in increased interest from other scientists to determine whether low dietary silicon may contribute to decreased bone strength and increased risk to fractures, and to delayed bone healing or repair. The findings also have stimulated the appearance of nutritional supplements that contain silicon. This accomplishment was conducted under National Program 107 Human Nutrition; ARS Strategic Plan goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining functions for emerging classes of nutrients.

Substitution of Soy Protein for Meat Protein Did Not Improve Bone Health in Postmenopausal Women:
The US Food and Drug Administration has approved a health claim that a daily consumption of 25 g of soy protein is beneficial to heart health. However, the effects of this dietary practice on bone health are unknown. A controlled feeding trial with postmenopausal women, using sensitive whole body counting methodology, demonstrated that when 25 g of soy protein is substituted for an equal weight of meat protein, on a daily basis for 8 weeks, the amount of calcium that the body retains does not change. Furthermore, urinary and blood biomarkers of bone formation and breakdown did not change. These findings indicate that the common practice of substitution of soy protein for meat protein does not provide any additional benefits (associated with phytoestrogens) or risks (associated with

phytate) with regards to calcium retention or bone health in postmenopausal women and that the effects of vegetable proteins versus animal proteins on bone health deserve further investigations. This accomplishment was conducted under National Program 107 Human Nutrition; ARS Strategic Plan goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining interactions for known classes of nutrients.

Dietary Boron May Help Reduce The Amount of Insulin Needed to Maintain Blood Glucose:
Two separate studies with healthy rats and a third with chicks were conducted to determine whether boron, a natural substance in the diet, helps regulate the level of insulin in the blood. Results from the rat studies demonstrated that a diet with normal physiological amounts of boron reduced blood insulin levels without affecting glucose levels. In chicks given glucose through a vein injected, boron decreased peak pancreatic insulin release. These results suggest that dietary boron, found in higher concentrations in fruits, vegetables, nuts, and legumes, may help reduce the amount of insulin needed to maintain blood glucose. This accomplishment was conducted under National Program 107 Human Nutrition; ARS Strategic Plan goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining functions for emerging classes of nutrients.

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**

Information about the nutritional of beneficial aspects of ultratrace and trace elements as it became available was routinely transferred to a variety of customers. The customers included nutritional risk assessment groups through direct contact or organized meetings and workshops; the public through web pages of professional organizations, via the popular media; and other scientists through presentations at national and international meetings and professional publications.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below).**

Information was transferred to the public through the local newspaper (Grand Forks Herald) that was also placed on the Grand Forks Human Nutrition Research Center Home Page. Dr. Roughead wrote an article entitled, "Vitamin D: you need it for good bones." Dr. Nielsen wrote an article entitled, "Don't overlook benefits of diet that contain fish."

Scientific Publications: **Log 115:**

1. Hunt, C., Friel, J.K., Johnson, L.K. 2004. Boron concentrations in milk from mothers of full-term and premature infants. American Journal of Clinical Nutrition. 80:1327-33. 0000158843
2. Nielsen, F.H., Poellot, R.A. 2004. Dietary silicon affects bone turnover differently in ovariectomized and sham-operated growing rats. Journal of Trace Elements in Experimental Medicine. 17:137-149. 0000149023
3. Nielsen, F.H. 2004. Dietary fat composition modifies the effect of boron on bone characteristics and plasma lipids in rats. Biofactors. 20(3):161-71. 0000160626
4. Roughead, Z.K., Hunt, J.R., Johnson, L.K., Badger, T.M., Lykken, G.I. 2005. Controlled substitution of soy protein for meat protein: effects on calcium retention, bone, and cardiovascular health indices in postmenopausal women. 0000159105

Project Number: 5450-51000-034-00D **Accession:** 0405069 **FY:** 2005

Journal of Clinical Endocrinology and Metabolism. 90(1):181-9.

5. Hunt, C. 2005. Boron. In: P.M. Coates, M.R. Blackman, G. Cragg, M. Levine, J. Moss, J. White, editors. Encyclopedia of Dietary Supplements. New York: Marcel Dekker/Taylor and Francis Group. p. 55-63. **0000185185**

6. Nielsen, F.H. 2004. The effect of nickel deprivation on bone strength and shape and urinary phosphorus excretion is not enhanced by a mild magnesium deprivation in rats. In: Anke, M., Flachowsky, G., Kisters, K., Schafer, U., Schenkel, H., Seifert, M., Stoeppler, M., editors. Proceedings of the Macro and Trace Elements 22nd Workshop, September 24-25, 2004, Jena, Germany. 2:965-70. **0000169181**

7. Roughead, Z.K. 2004. Member Spotlight. The Digest. Newsletter of the Research Dietetic Practice Group of the American Dietetic Association. 39(4):8-9. **0000168031**

8. Combs Jr., G.F., Hassan, N., Hunt, C.D., Watts, J. 2005. Apparent efficacy of food-based calcium supplementation in preventing rickets in Bangladesh [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1462. **0000171715**

9. Durick, K.A., Tomita, M., Hunt, C., Bradley, D. 2005. Evidence that boron down-regulates inflammation through the NF-KB pathway [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1705. **0000171287**

10. Durick, K.A., Hunt, C., Bradley, D. 2005. Evidence for anti-inflammatory properties of dietary boron in collagen-immunized B10.T(6R), an animal model of polyarthritis [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(4):A915. **0000171397**

11. Hunt, C. 2004. Dietary boron as a factor in glucose and insulin metabolism [abstract]. The Journal of Trace Elements in Experimental Medicine. 17(4):258. **0000166092**

12. Hunt, C.D., Butte, N.F. 2005. Boron concentrations remain stable in milk from mothers of full-term exclusively breast-fed infants during the first four months of lactation [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1704. **0000171367**

13. Nielsen, F.H. 2004. Essentiality of copper, zinc, magnesium, boron and silicon in bone development and function [abstract]. The Journal of Trace Elements in Experimental Medicine. 17(4):263-4. **0000165659**

14. Nielsen, F.H. 2005. Fish oil instead of safflower oil as the dietary fat source modifies the oxidative stress response to boron deficiency in rats [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1705. **0000171281**

15. Stoecker, B.J., Nielsen, F.H. 2005. Dietary fatty acid composition, dietary boron, and ovariectomy affect bone strength and microarchitecture [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(4):A57. **0000175002**

16. Rhee, Y., Hunt, C., Idso, J.P. 2004. Dietary boron reduces serum insulin concentrations regardless of vitamin d status in rats [abstract]. The Journal of Trace Elements in Experimental Medicine. 17(4):208. **0000165653**

17. Roughead, Z.K., Johnson, L.K., Lykken, G.I. 2004. The effects of interaction of dietary protein and calcium on calcium retention: a controlled feeding study [abstract]. Journal of Bone and Mineral Research. 19(sup1):S302. **0000164955**

18. Roughead, Z.K. 2004. Dairy protein and bone health [abstract]. International Dairy Federation (IDF) World Dairy Summit. p.47. **0000170328**

Project Number: 5450-51000-034-00D **Accession:** 0405069 **FY:** 2005

19. Roughead, Z.K., Johnson, L.K., Lykken, G.I. 2005. A high protein intake enhances calcium retention from a low calcium diet in healthy postmenopausal women: A controlled feeding study [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1463. **0000171282**

20. Sattler, J.A., Soule, M.R., Hillegonds, D.J., Roughead, Z.K., Wagner, J.L. 2005. Whole body versus skeletal calcium (Ca) retention in rats: Short and long term comparisons using 47CA and 41Ca tracers [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(4):A60. **0000171593**

21. Guglielmotti, M.B., Gorustovich, A., Krieger, L., Renou, S.J., Giglio, M.J., Nielsen, F.H. 2005. Bone healing under a silicon-deficient diet: a histomorphometric study in rats [abstract]. Presented at the Annual Meeting of the International Association for Dental Research, Baltimore, MD. March 9 - 12, 2005. Journal Dental Research 84(Spec Iss A):abstract 2157. **0000174893**

Approved: CHANDLER LAURENCE D **Date:** 09/20/2005

Project Number: 5450-51000-034-02T **Accession:** 0407132 **FY:** 2005
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH
Start Date: 02/01/2003 **Term Date:** 05/31/2005

National Programs: 107 N Human Nutrition

Title: MEAT PROTEIN AND CALCIUM: DO THEY INTERACT SYNERGISTICALLY OR ANTAGONISTICALLY?

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 58-5450-3-0410

Organization Name: NATIONAL CATTLEMEN'S BEEF ASSOCIATION

Progress and Outcomes:

4d. **Progress report.**

This report serves to document research conducted under trust agreement #58-5450-3-410 between ARS and the National Cattleman's Beef Association. Additional details of this project can be found in the report for the parent CRIS 5450-510000-034-00D.

To better define the relationship between dietary calcium and meat protein, we have successfully conducted a carefully controlled feed study in 27 postmenopausal women. The findings were summarized in the parent CRIS and were reported at the 2004 Annual meeting of The American Society for Bone and Mineral Research in Seattle, Washington in October, 2004.

Scientific Publications: **Log 115:**

Approved: CHANDLER LAURENCE D **Date:** 10/03/2005

Project Number: 5450-51000-034-04T **Accession:** 0407514 **FY:** 2005
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH
Start Date: 10/01/2001 **Term Date:** 03/01/2005

National Programs: 107 N Human Nutrition

Title: EFFECTS OF PROTEIN ON CALCIUM RETENTION AND BONE METABOLISM IN POSTMENOPAUSAL WOMEN

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 58-5450-2-0401

Organization Name: NORTH DAKOTA BEEF COMMISSION

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

None

4b. List other significant accomplishments, if any.

None

4c. List any significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under Trust Fund Cooperative Agreement #58-5450-2-0401 between ARS and North Dakota Beef Commission. Additional details of the research can be found in the reports for the parent CRIS project 5450-51000-034-00D, Mineral Intakes for Optimal Bone and Joint Development and Health.

During the life of this project we have found that when healthy postmenopausal women consume 25 g of soy protein in place of an equivalent amount of meat protein for several weeks, calcium retention and measures of bone and cardiovascular health are not affected. This is a significant finding as animal protein is often cited as a risk factor for debilitating chronic diseases such as osteoporosis and heart disease. A manuscript summarizing the results was published in the J. Clinical Endocrinology and Metabolism.

As a follow-up to this project, we conducted a carefully controlled feeding study in 27 postmenopausal women. The findings were summarized in the parent CRIS and were reported at the 2004 Annual meeting of The American Society for Bone and Mineral Research in Seattle, Washington in October, 2004.

Scientific Publications: **Log 115:**

Approved: CHANDLER LAURENCE D **Date:** 09/27/2005

Project Number: 5450-51000-035-04T **Accession:** 0402795 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: MARY J KRETSCH
Start Date: 02/22/2000 **Term Date:** 02/21/2005
National Programs: 107 N Human Nutrition
Title: HEALTH BENEFITS OF FOOD FORMS OF SELENIUM
Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** Yes
Terminate in Two Months? No
Agreement Number: 58-3K95-0-0813
Organization Name: GENERAL MILLS

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

Products made from selenium-enriched whole wheat may be a means of supplementing dietary selenium intakes, but there are few reports in the literature of the bioavailability of selenium from processed wheat. A human study fed for 3 months to healthy men a single serving of whole-wheat breakfast cereal; the cereal was made from wheat with either high or low concentrations of selenium and provided either less than 50 or more than 250 micrograms of selenium/day. The selenium was bioavailable as demonstrated by an increase in plasma selenium concentrations. Intense exercise was used to induce oxidative stress, and results of physiologic tests suggested that subjects consuming the high-selenium wheat cereal had improved oxygen consumption and decreased oxidative stress. These results demonstrate that high-selenium wheat cereal may be an effective means of supplementing selenium to the general population.

4b. List other significant accomplishments, if any.

Selenium supplementation may enhance the immune system and decrease inflammation. Rats were fed diets that included whole-wheat breakfast cereal made from either high-selenium or low-selenium wheat. Rats fed the high-selenium wheat cereal had an improved tolerance to a repeated exposure to an adjuvant challenge and exhibited less swelling in an adjuvant-induced arthritis rat model. These data suggest that selenium from wheat is effective for reduction of chronic and acute inflammation.

4c. List any significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under Cooperative Research and Development Agreement number 58-3K95-0-0813 between ARS and General Mills, Inc. Additional details of this research can be found in the report for the parent CRIS 5450-51000-035-00D.

Scientific Publications: **Log 115:**

1. Finley, J.W. 2005. Whole-wheat cereal improves fermentation in healthy men [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A984. 0000171625

Approved: CHANDLER LAURENCE D **Date:** 10/11/2005

Project Number: 5450-51000-035-05T **Accession:** 0403407 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH

Start Date: 09/01/2000 **Term Date:** 08/31/2005

National Programs: 107 N Human Nutrition

Title: HIGH SELENIUM MEAT, WHEAT, AND BROCCOLI: A MARKETABLE ASSET?

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 01-5450-3-0157

Organization Name: **INITIATIVE FOR FUTURE AGRICULTURE AND FOOD SYSTEMS (IFAFS)DEPARTMENT OF AGRICULTURE, CSREES**

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

Wheat enriched in selenium may be a means of supplementing dietary selenium intakes. However, one of the primary reasons for consuming excess selenium is to inhibit development of cancer, and there is little information as to whether selenium from wheat is effective for cancer reduction. An initial experiment by our laboratory demonstrated that selenium from wheat reduced the incidence of aberrant crypts, preneoplastic markers of colon cancer. However a series of five follow-up experiments failed to confirm this finding. Consequently, we have concluded that while selenium from wheat is highly bioavailable for activation of selenoproteins and increasing tissue selenium status, there is not evidence to support the hypothesis that selenium from wheat is effective for the reduction of colon cancer.

4b. List other significant accomplishments, if any.

None

4c. List any significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under reimbursable agreement number 01-5450-3-0157 between ARS and the Initiative for Future Agricultural and Food Systems, CREES/USDA. In addition to work conducted at the GFHNRC, money from this grant has been transferred to the following projects: 5450-51000-035: -06S, -07G, -08S, -09S, -010G, -11S. For complete details of work accomplished under this project, annual reports detailing work conducted in the above agreements also should be consulted. Additional details of this research can be found in the report for the parent CRIS 5450-51000-035-00D.

Scientific Publications: **Log 115:**

1. Finley, J.W., Keck, A.S., Robbins, R.J., Hintze, K.J. 2005. Selenium enrichment of broccoli: interactions between selenium and secondary plant compounds. Journal of Nutrition. 135:1236-38. **0000168017**

2. Finley, J.W. 2005. Proposed criteria for assessing the efficacy of cancer reduction by plant foods enriched in carotenoids, glucosinolates, polyphenols and selenocompounds. Annals of Botany. 95:1075-96. **0000174597**

Project Number: 5450-51000-035-05T **Accession:** 0403407 **FY:** 2005

3. Keck, A-S., Finley, J.W. 2005. Broccoli improved lipid values and selenium-enriched broccoli increased plasma selenium in healthy males [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1454. **0000171595**

4. Eberhardt, M.V., Kobira, K., Keck, A.S., Finley, J.W., Juvik, J.A., Jeffery, E.H. 2005. Correlations of chemical composition, antioxidant capacity and cellular oxidative stress in extracts from broccoli cultivars [abstract]. The Federation of American Societies for Experimental Biology Journal. 19(5):A1475. **0000171594**

5. Robbins, R.J., Keck, A., Banuelos, G.S., Finley, J.W. 2005. Cultivation conditions and selenium fertilization alter the phenolic profile, glucosinolate and sulforaphane content of broccoli. Journal of Medicinal Food. 8:204-214. **0000163665**

Approved: CHANDLER LAURENCE D **Date:** 10/03/2005

MICRONUTRIENT ABSORPTION AND METABOLISM

MANAGEMENT UNIT

5450-020-00

Project Number: 5450-51000-035-14T **Accession:** 0405700 **FY:** 2005
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH

Start Date: 06/01/2002 **Term Date:** 09/30/2004

National Programs: 107 N Human Nutrition

Title: CHANGING THE NUTRITIONAL COMPOSITION OF BEEF: CAN WE MITIGATE THE RISK OF COLON CANCER?

Period Covered **From:** 10/ 2004 **To:** 9/ 2005 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 58-5450-2-0421

Organization Name: **NATIONAL CATTLEMEN'S BEEF ASSOCIATION**

Progress and Outcomes:

4a. What was the single most significant accomplishment this past year?

Beef raised on high selenium pasture or fed high-selenium feedstuffs in a feedlot may accumulate selenium in concentrations 10-20 fold higher than average. Such beef may be an ideal means of supplementing selenium to humans desirous of increasing their selenium intake. A human study fed high-selenium or low-selenium beef to healthy adult men for 3 months. The selenium from beef was bioavailable as determined by an increase in plasma selenium concentrations. Gene arrays were used to assess gene expression in leucocytes; analysis of the gene arrays is ongoing. Preliminary data from this study suggests that high-selenium beef is an excellent means of supplementing selenium to humans.

4b. List other significant accomplishments, if any.

None

4c. List any significant activities that support special target populations.

None

4d. Progress report.

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